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(54) **NANBV diagnostics**

NANBV-Diagnostika

Diagnostics de NANBV

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- **PROC. NATL. ACAD. SCI. USA**, vol. 84, September 1987, pp. 6277-6281, D.W. BRADLEY et al.: "Enterically transmitted non-A, non-B hepatitis: Serial passage of disease in cynomolgus macaques and tamarins and recovery of disease-associated 27- to 34-nm viruslike particles"
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## Description

## Technical Field

- 5 [0001] The invention relates to materials and methodologies for managing the spread of non-A, non-B hepatitis virus (NANBV) infection. More specifically, it relates to diagnostic DNA fragments, diagnostic proteins, diagnostic antibodies and protective antigens and antibodies for an etiologic agent of NANB hepatitis, i.e., hepatitis C virus.

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**[0003]**

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 50 U.S. Patent No. 4,493,890

Background Art

- [0004]** Non-A, Non-B hepatitis (NANBH) is a transmissible disease or family of diseases that are believed to be viral-  
 55 induced, and that are distinguishable from other forms of viral-associated liver diseases, including that caused by the  
 known hepatitis viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), and delta hepatitis virus (HDV), as well  
 as the hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH was first identified in transfused  
 individuals. Transmission from man to chimpanzee and serial passage in chimpanzees provided evidence that NANBH

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is due to a transmissible infectious agent or agents. However, the transmissible agent responsible for NANBH is still unidentified and the number of agents which are causative of the disease are unknown.

[0005] Epidemiologic evidence is suggestive that there may be three types of NANBH: the water-borne epidemic type; the blood or needle associated type; and the sporadically occurring (community acquired) type. However, the number of agents which may be the causative of NANBH are unknown.

[0006] Clinical diagnosis and identification of NANBH has been accomplished primarily by exclusion of other viral markers. Among the methods used to detect putative NANBV antigens and antibodies are agar-gel diffusion, counter-immunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay. However, none of these assays has proved to be sufficiently sensitive, specific, and reproducible to be used as a diagnostic test for NANBH.

[0007] Until now there has been neither clarity nor agreement as to the identity or specificity of the antigen antibody systems associated with agents of NANBH. This is due, at least in part, to the prior or co-infection of HBV with NANBV in individuals, and to the known complexity of the soluble and particulate antigens associated with HBV, as well as to the integration of HBV DNA into the genome of liver cells. In addition, there is the possibility that NANBH is caused by more than one infectious agent, as well as the possibility that NANBH has been misdiagnosed. Moreover, it is unclear what the serological assays detect in the serum of patients with NANBH. It has been postulated that the agar-gel diffusion and counterimmunoelectrophoresis assays detect autoimmune responses or non-specific protein interactions that sometimes occur between serum specimens, and that they do not represent specific NANBV antigen-antibody reactions. The immunofluorescence, and enzyme-linked immunosorbent, and radioimmunoassays appear to detect low levels of a rheumatoid-factor-like material that is frequently present in the serum of patients with NANBH as well as in patients with other hepatic and nonhepatic diseases. Some of the reactivity detected may represent antibody to host-determined cytoplasmic antigens.

[0008] There are a number of candidate NANBV. See, for example the reviews by Prince (1983), Feinstone and Hoofnagle (1984), and Overby (1985, 1986, 1987) and the article by Iwarson (1987). However, there is no proof that any of these candidates represent the etiologic agent of NANBH.

[0009] The demand for sensitive, specific methods for screening and identifying carriers of NANBV and NANBV contaminated blood or blood products is significant. Post-transfusion hepatitis (PTH) occurs in approximately 10% of transfused patients, and NANBH accounts for up to 90% of these cases. The major problem in this disease is the frequent progression to chronic liver damage (25-55%).

[0010] Patient care as well as the prevention of transmission of NANBH by blood and blood products or by close personal contact require reliable diagnostic and prognostic tools to detect nucleic acids, antigens and antibodies related to NANBV. In addition, there is also a need for effective vaccines and immunotherapeutic therapeutic agents for the prevention and/or treatment of the disease.

#### Disclosure of the Invention

[0011] The invention pertains to the isolation and characterization of a newly discovered etiologic agent of NANBH, hepatitis C virus (HCV). More specifically, the invention provides a family of cDNA replicas of portions of HCV genome. These cDNA replicas were isolated by a technique which included a novel step of screening expression products from cDNA libraries created from a particulate agent in infected tissue with sera from patients with NANBH to detect newly synthesized antigens derived from the genome of the heretofore unisolated and uncharacterized viral agent, and of selecting clones which produced products which reacted immunologically only with sera from infected individuals as compared to non-infected individuals.

[0012] Studies of the nature of the genome of the HCV, utilizing probes derived from the HCV cDNA, as well as sequence information contained within the HCV cDNA, are suggestive that HCV is a Flavivirus or a Flavi-like virus.

[0013] Portions of the cDNA sequences derived from HCV are useful as probes to diagnose the presence of virus in samples, and to isolate naturally occurring variants of the virus. These cDNAs also make available polypeptide sequences of HCV antigens encoded within the HCV genome(s) and permits the production of polypeptides which are useful as standards or reagents in diagnostic tests and/or as components of vaccines. Antibodies, both polyclonal and monoclonal, directed against HCV epitopes contained within these polypeptide sequences are also useful for diagnostic tests, as therapeutic agents, for screening of antiviral agents, and for the isolation of the NANBV agent from which these cDNAs derive. In addition, by utilizing probes derived from these cDNAs it is possible to isolate and sequence other portions of the HCV genome, thus giving rise to additional probes and polypeptides which are useful in the diagnosis and/or treatment, both prophylactic and therapeutic, of NANBH.

[0014] Thus, the invention provides a polymerase chain reaction (PCR) kit comprising a pair of primers capable of priming the synthesis of cDNA in a PCR reaction, wherein each or said primers is a polynucleotide comprising a contiguous sequence or nucleotides which is capable of selectively hybridizing to the genome of hepatitis C virus (HCV) or the complement thereof, wherein HCV is characterized by:

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a positive stranded RNA genome;

said genome comprising an open reading frame (ORF) encoding a polypeptide; and

5 the entirety of the said encoded polypeptide having at least 40% homology to the entire polypeptide or a viral isolate from the genome or which was prepared cDNAs deposited in a lambda gt-11 cDNA library with the American Type Culture Collection (ATCC) under accession no. 40394.

10 [0015] The invention also provides a method of performing a polymerase chain reaction wherein the primers are a pair of polynucleotides as defined above in relation to PCR kits of the invention.

[0016] The invention also provides a method for assaying a sample for the presence or absence of HCV polynucleotides comprising:

15 (a) contacting the sample with a probe under conditions that allow the selective hybridisation of said probe to an HCV polynucleotide or the complement thereof in the sample, wherein said probe comprises a polynucleotide comprising a contiguous sequence of nucleotides which is capable of selectively hybridising to the genome of HCV or the complement thereof, wherein HCV is characterised by:

20 (i) a positive stranded RNA genome, said genome comprising an open reading frame (ORF) encoding a polypeptide; and  
(ii) the entirety of the said encoded polypeptide having at least 40% homology to the entire polypeptide of a viral isolate from the genome of which was prepared cDNAs deposited in a lambda gt-11 cDNA library with the American Type Culture Collection (ATCC) under accession no. 40394;

25

and

(b) determining whether polynucleotide duplexes comprising said probe are formed

and further wherein said polynucleotide is a DNA polynucleotide and optionally comprises a detectable label.

30 [0017] We also describe a purified HCV polynucleotide; a recombinant HCV polynucleotide; a recombinant polynucleotide comprising a sequence derived from an HCV genome or from HCV cDNA; a recombinant polynucleotide encoding an epitope of HCV; a recombinant vector containing any of the above recombinant polynucleotides, and a host cell transformed with any of these vectors.

35 [0018] We also describe a recombinant expression system comprising an open reading frame (ORF) of DNA derived from an HCV genome or from HCV cDNA, wherein the ORF is operably linked to a control sequence compatible with a desired host, a cell transformed with the recombinant expression system, and a polypeptide produced by the transformed cell.

40 [0019] It is also possible to obtain purified HCV particles, a preparation of polypeptides from the purified HCV; a purified HCV polypeptide; a purified polypeptide comprising an epitope which is immunologically identifiable with an epitope contained in HCV.

[0020] We also describe a recombinant HCV polypeptide; a recombinant polypeptide comprised of a sequence derived from an HCV genome or from HCV cDNA; a recombinant polypeptide comprised of an HCV epitope; and a fusion polypeptide comprised of an HCV polypeptide.

45 [0021] We also describe an anti-HCV antibody composition comprising antibodies that bind said antigenic determinant of a polypeptide according to the invention which is (a) a purified preparation of polyclonal antibodies, or (b) a monoclonal antibody composition.

50 [0022] We also describe a particle which is immunogenic against HCV infection comprising a non-HCV polypeptide having an amino acid sequence capable of forming a particle when said sequence is produced in a eukaryotic host, and an HCV epitope. The invention also relates to a polynucleotide probe for HCV, the probe comprising a polynucleotide of the invention which further comprises a detectable label. The invention also relates to a polymerase chain reaction (PCR) kit comprising a pair of primers capable of priming the synthesis of cDNA in a PCR reaction where each of the primers is a polynucleotide according to the invention. The invention also finds application in the production of kits such as those for assaying a sample for the presence or absence of HCV polynucleotides by (a) contacting the sample with a probe comprising a polynucleotide of the invention, for example one containing about 8 or more nucleotides, under conditions that allow the selective hybridization of said probe to an HCV polynucleotide or the complement thereof in the sample; and (b) detecting any polynucleotide duplexes comprising said probe.

55 [0023] We also describe a polypeptide comprised of an HCV epitope, attached to a solid substrate; and an antibody to an HCV epitope, attached to a solid substrate,

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[0024] We also describe a method for producing a polypeptide containing an HCV epitope comprising incubating host cells transformed with an expression vector containing a sequence encoding a polypeptide containing an HCV epitope under conditions which allow expression of said polypeptide; and a polypeptide containing an HCV epitope produced by this method.

5 [0025] The invention also relates to a method for detecting HCV nucleic acids in a sample comprising reacting nucleic acids of the sample with a probe for an HCV polynucleotide under conditions which allow the formation of a polynucleotide duplex between the probe and the HCV nucleic acid from the sample; and detecting a polynucleotide duplex which contains the probe.

[0026] We also describe immunoassays. These include an immunoassay for detecting an HCV antigen comprising (a) providing an antibody composition according to the invention; (b) incubating a sample with the antibody composition under conditions that allow for the formation of an antibody-antigen complex; and (c) detecting antibody-antigen complexes comprising the anti-HCV antibodies. These also include an immunoassay for detecting antibodies directed against an HCV antigen comprising (a) providing a polypeptide comprising an antigenic determinant bindable by said anti-HCV antibody, wherein said antigenic determinant comprises a contiguous amino acid sequence encoded by said genome; (b) incubating a biological sample with said polypeptide under conditions that allow for the formation of an antibody-antigen complex; and (c) detecting antibody-antigen complexes comprising said polypeptide.

[0027] We also describe vaccine compositions for treatment of HCV infection comprising an immunogenic peptide containing an HCV epitope, or an inactivated preparation of HCV, or an attenuated preparation of HCV.

[0028] We also describe a tissue culture grown cell infected with HCV and the invention includes a method of growing HCV by providing cells, e.g. hepatocytes or macrophages, infected with HCV and propagating such cells in vitro.

[0029] We also describe a method for producing antibodies to HCV comprising administering to an individual an isolated immunogenic polypeptide containing an HCV epitope in an amount sufficient to induce an immune response.

[0030] We also describe a method for isolating cDNA derived from the genome of an unidentified infectious agent, comprising: (a) providing host cells transformed with expression vectors containing a cDNA library prepared from nucleic acids isolated from tissue infected with the agent and growing said host cells under conditions which allow expression of polypeptide(s) encoded in the cDNA; (b) interacting the expression products of the cDNA with an antibody containing body component of an individual infected with said infectious agent under conditions which allow an immunoreaction, and detecting antibody-antigen complexes formed as a result of the interacting; (c) growing host cells which express polypeptides that form antibody-antigen complexes in step (b) under conditions which allow their growth as individual clones and isolating said clones; (d) growing cells from the clones of (c) under conditions which allow expression of polypeptide(s) encoded within the cDNA, and interacting the expression products with antibody containing body components of individuals other than the individual in step (a) who are infected with the infectious agent and with control individuals uninfected with the agent, and detecting antibody-antigen complexes formed as a result of the interacting; (e) growing host cells which express polypeptides that form antibody-antigen complexes with antibody containing body components of infected individuals and individuals suspected of being infected, and not with said components of control individuals, under conditions which allow their growth as individual clones and isolating said clones; and (f) isolating the cDNA from the host cell clones of (e).

#### Brief Description of the Drawings

40 [0031] Fig. 1 shows the double-stranded nucleotide sequence of the HCV cDNA insert in clone 5-1-1, and the putative amino acid sequence of the polypeptide encoded therein.

[0032] Fig. 2 shows the homologies of the overlapping HCV cDNA sequences in clones 5-1-1, 81, 1-2, and 91.

45 [0033] Fig. 3 shows a composite sequence of HCV cDNA derived from overlapping clones 81, 1-2, and 91, and the amino acid sequence encoded therein.

[0034] Fig. 4 shows the double-stranded nucleotide sequence of the HCV cDNA insert in clone 81, and the putative amino acid sequence of the polypeptide encoded therein.

[0035] Fig. 5 shows the HCV cDNA sequence in clone 36, the segment which overlaps the NANBV cDNA of clone 81, and the polypeptide sequence encoded within clone 36.

50 [0036] Fig. 6 shows the combined ORF of HCV cDNAs in clones 36 and 81, and the polypeptide encoded therein.

[0037] Fig. 7 shows the HCV cDNA sequence in clone 32, the segment which overlaps clone 81, and the polypeptide encoded therein.

[0038] Fig. 8 shows the HCV cDNA sequence in clone 35, the segment which overlaps clone 36, and the polypeptide encoded therein.

55 [0039] Fig. 9 shows the combined ORF of HCV cDNAs in clones 35, 36, 81, and 32, and the polypeptide encoded therein.

[0040] Fig. 10 shows the HCV cDNA sequence in clone 37b, the segment which overlaps clone 35, and the polypeptide encoded therein.

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- [0041] Fig. 11 shows the HCV cDNA sequence in clone 33b, the segment which overlaps clone 32, and the polypeptide encoded therein.
- [0042] Fig. 12 shows the HCV cDNA sequence in clone 40b, the segment which overlaps clone 37b, and the polypeptide encoded therein.
- 5 [0043] Fig. 13 shows the HCV cDNA sequence in clone 25c, the segment which overlaps clone 33b, and the polypeptide encoded therein.
- [0044] Fig. 14 shows the nucleotide sequence and polypeptide encoded therein of the ORF which extends through the HCV cDNAs in clones 40b, 37b, 35, 36, 81, 32, 33b, and 25c.
- [0045] Fig. 15 shows the HCV cDNA sequence in clone 33c, the segment which overlaps clones 40b and 33c, and the amino acids encoded therein.
- 10 [0046] Fig. 16 shows the HCV cDNA sequence in clone 8h, the segment which overlaps clone 33c, and the amino acids encoded therein.
- [0047] Fig. 17 shows the HCV cDNA sequence in clone 7e, the segment which overlaps clone 8h, and the amino acids encoded therein.
- 15 [0048] Fig. 18 shows the HCV cDNA sequence in clone 14c, the segment which overlaps clone 25c, and the amino acids encoded therein.
- [0049] Fig. 19 shows the HCV cDNA sequence in clone 8f, the segment which overlaps clone 14c, and the amino acids encoded therein.
- [0050] Fig. 20 shows the HCV cDNA sequence in clone 33f, the segment which overlaps clone 8f, and the amino acids encoded therein.
- 20 [0051] Fig. 21 shows the HCV cDNA sequence in clone 33g, the segment which overlaps clone 33f, and the amino acids encoded therein.
- [0052] Fig. 22 shows the HCV cDNA sequence in clone 7f, the segment which overlaps the sequence in clone 7e, and the amino acids encoded therein.
- 25 [0053] Fig. 23 shows the HCV cDNA sequence in clone 11b, the segment which overlaps the sequence in clone 7f, and the amino acids encoded therein..
- [0054] Fig. 24 shows the HCV cDNA sequence in clone 14i, the segment which overlaps the sequence in clone 11b, and the amino acids encoded therein.
- [0055] Fig. 25 shows the HCV cDNA sequence in clone 39c, the segment which overlaps the sequence in clone 33g, and the amino acids encoded therein.
- 30 [0056] Fig. 26 shows a composite HCV cDNA sequence derived from the aligned cDNAs in clones 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g and 39c also shown is the amino acid sequence of the polypeptide encoded in the extended ORF in the derived sequence.
- [0057] Fig. 27 shows the sequence of the HCV cDNA in clone 12f, the segment which overlaps clone 14i, and the amino acids encoded therein.
- 35 [0058] Fig. 28 shows the sequence of the HCV cDNA in clone 35f, the segment which overlaps clone 39c, and the amino acids encoded therein.
- [0059] Fig. 29 shows the sequence of the HCV cDNA in clone 19g, the segment which overlaps clone 35f, and the amino acids encoded therein.
- 40 [0060] Fig. 30 shows the sequence of clone 26g, the segment which overlaps clone 19g, and the amino acids encoded therein.
- [0061] Fig. 31 shows the sequence of clone 15e, the segment which overlaps clone 26g, and the amino acids encoded therein.
- [0062] Fig. 32 shows the sequence in a composite cDNA, which was derived by aligning clones 12f through 15e in the 5' to 3' direction; it also shows the amino acids encoded in the continuous ORF.
- 45 [0063] Fig. 33 shows a photograph of Western blots of a fusion protein, SOD-NANB<sub>5-1-1</sub>, with chimpanzee serum from chimpanzees infected with BB-NANB, HAV, and HBV.
- [0064] Fig. 34 shows a photograph of Western blots of a fusion protein, SOD-NANB<sub>5-1-1</sub>, with serum from humans infected with NANBV, HAV, HBV, and from control humans.
- 50 [0065] Fig. 35 is a map showing the significant features of the vector pAB24.
- [0066] Fig. 36 shows the putative amino acid sequence of the carboxy-terminus of the fusion polypeptide C100-3 and the nucleotide sequence encoding it.
- [0067] Fig. 37A is a photograph of a coomassie blue stained polyacrylamide gel which identifies C100-3 expressed in yeast
- 55 [0068] Fig. 37B shows a Western blot of C100-3 with serum from a NANBV infected human.
- [0069] Fig. 38 shows an autoradiograph of a Northern blot of RNA isolated from the liver of a BB-NANBV infected chimpanzee, probed with BB-NANBV cDNA of clone 81.
- [0070] Fig. 39 shows an autoradiograph of NANBV nucleic acid treated with RNase A or DNase I, and probed with

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BB-NANBV cDNA of clone 81.

[0071] Fig. 40 shows an autoradiograph of nucleic acids extracted from NANBV particles captured from infected plasma with anti-NANB<sub>5-1-1</sub>, and probed with <sup>32</sup>P-labeled NANBV cDNA from clone 81.

[0072] Fig. 41a and b shows autoradiographs of filters containing isolated NANBV nucleic acids, probed with <sup>32</sup>P-labeled plus and minus strand DNA probes derived from NANBV cDNA in clone 81.

[0073] Fig. 41-1 shows the homologies between a polypeptide encoded in HCV cDNA and an NS protein from Dengue flavivirus.

[0074] Fig. 43 shows a histogram of the distribution of HCV infection in random samples, as determined by an ELISA screening.

[0075] Fig. 44 shows a histogram of the distribution of HCV infection in random samples using two configurations of immunoglobulin-enzyme conjugate in an ELISA assay.

[0076] Fig. 45 shows the sequences in a primer mix, derived from a conserved sequence in NS1 of flaviviruses.

[0077] Fig. 46 shows the HCV cDNA sequence in clone k9-1, the segment which overlaps the cDNA in Fig. 27, and the amino acids encoded therein.

[0078] Fig. 47 shows the sequence in a composite CDNA which was derived by aligning clones k9-1 through 15e in the 5' to 3' direction; it also shows the amino acids encoded in the continuous ORF.

#### 1. Definitions

[0079] The term "hepatitis C virus" has been reserved by workers in the field for an heretofore unknown etiologic agent of NANBH. Accordingly, as used herein, "hepatitis C virus" (HCV) refers to an agent causative of NANBH, which agent is a virus characterised by

a positive stranded RNA genome;

said genome comprising an open reading frame (ORF) encoding a polyprotein; and

the entirety of the said encoded polyprotein having at least 40% homology to the entire polyprotein of a viral isolate from the genome of which was prepared cDNAs deposited in a lambda gt-11 CDNA library with the American Type Culture Collection (A-TCC) under accession no. 40394.

[0080] This agent was formerly referred to as NANBV and/or BB-NANBV. The terms HCV, NANBV, and BB-NANBV are used interchangeably herein, but all refer to the virus as defined above. As an extension of this terminology, the disease caused by HCV, formerly called NANB hepatitis (NANBH), is called hepatitis C. The terms NANBH and hepatitis C may be used interchangeably herein.

[0081] The term "HCV", as used herein, denotes a viral species which causes NANBH, and attenuated strains or defective interfering particles derived therefrom. As shown infra., the HCV genome is comprised of RNA. It is known that RNA containing viruses have relatively high rates of spontaneous mutation, i.e., reportedly on the order of 10<sup>-3</sup> to 10<sup>-4</sup> per nucleotide (Fields & Knipe (1986)). Therefore, there are multiple strains within the HCV species described infra. The compositions and methods described herein, enable the propagation, identification, detection, and isolation of the various related strains. Moreover, they also allow the preparation of diagnostics and vaccines for the various strains, and have utility in screening procedures for anti-viral agents for pharmacologic use in that they inhibit replication of HCV.

[0082] The information provided herein, although derived from one strain of HCV, hereinafter referred to as CDC/HCV1, is sufficient to allow a viral taxonomist to identify other strains which fall within the species. As described herein, we have discovered that HCV is a Flavivirus or Flavi-like virus. The morphology and composition of Flavivirus particles are known, and are discussed in Brinton (1986). Generally, with respect to morphology, Flaviviruses contain a central nucleocapsid surrounded by a lipid bilayer. Virions are spherical and have a diameter of about 40-50 nm. Their cores are about 25-30 nm in diameter. Along the outer surface of the virion envelope are projections that are about 5-10 nm long with terminal knobs about 2 nm in diameter.

[0083] HCV encodes an epitope which is immunologically identifiable with an epitope in the HCV genome from which the cDNAs described herein are derived; preferably the epitope is encoded in a cDNA described herein. The epitope is unique to HCV when compared to other known Flaviviruses. The uniqueness of the epitope may be determined by its immunological reactivity with HCV and lack of immunological reactivity with other Flavivirus species. Methods for determining immunological reactivity are known in the art, for example, by radioimmunoassay, by Elisa assay, by hemagglutination, and several examples of suitable techniques for assays are provided herein.

[0084] In addition to the above, the following parameters are applicable, either alone or in combination, in identifying a strain as HCV. Since HCV strains are evolutionarily related, it is expected that the overall homology of the genomes

PCL XL error

Subsystem: IMAGE

Error: MissingData

Operator: ReadImage

Position: 8083

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rylations and the like.

[0107] "Transformation", as used herein, refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, for example, direct uptake, transduction, or f-mating. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

[0108] "Treatment" as used herein refers to prophylaxis and/or therapy.

[0109] An "individual", as used herein, refers to vertebrates, particularly members of the mammalian species, and includes but is not limited to domestic animals, sports animals, primates, and humans.

[0110] As used herein, the "plus strand" of a nucleic acid contains the sequence that encodes the polypeptide. The "minus strand" contains a sequence which is complementary to that of the "plus strand".

[0111] As used herein, a "positive stranded genome" of a virus is one in which the genome, whether RNA or DNA, is single-stranded and which encodes a viral polypeptide(s). Examples of positive stranded RNA viruses include Togaviridae, Coronaviridae, Retroviridae, Picornaviridae, and Caliciviridae. Included also, are the Flaviviridae, which were formerly classified as Togaviridae. See Fields & Knipe (1986).

[0112] As used herein, "antibody containing body component" refers to a component of an individual's body which is a source of the antibodies of interest. Antibody containing body components are known in the art, and include but are not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external secretions of the respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

[0113] As used herein, "purified HCV" refers to a preparation of HCV which has been isolated from the cellular constituents with which the virus is normally associated, and from other types of viruses which may be present in the infected tissue. The techniques for isolating viruses are known to those of skill in the art, and include, for example, centrifugation and affinity chromatography; a method of preparing purified HCV is discussed infra.

## II. Description

[0114] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See e.g., Maniatis, Fritsch & Sambrook, MOLECULAR CLONING; A LABORATORY MANUAL (1982); DNA CLONING, VOLUMES I AND II (D.N. Glover ed. 1985); OLIGONUCLEOTIDE SYNTHESIS (M.J. Gait ed. 1984); NUCLEIC ACID HYBRIDIZATION (B.D. Hames & S.J. Higgins eds. 1984); TRANSCRIPTION AND TRANSLATION (B.D. Hames & S.J. Higgins eds. 1984); ANIMAL CELL CULTURE (R.I. Freshney ed. 1986); IMMOBILIZED CELLS AND ENZYMES (IRL Press, 1986); B. Perbal, A PRACTICAL GUIDE TO MOLECULAR CLONING (1984);

the series; METHODS IN ENZYMOLOGY (Academic Press, Inc.);

GENE TRANSFER VECTORS FOR MAMMALIAN CELLS (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory), Methods in Enzymology Vol. 154 and Vol. 155 (Wu and Grossman; and Wu, eds., respectively), Mayer and Walker, eds. (1987) IMMUNOCHEMICAL METHODS IN CELL AND MOLECULAR BIOLOGY (Academic Press, London), Scopes, (1987), PROTEIN PURIFICATION: PRINCIPLES AND PRACTICE, Second Edition (Springer-Verlag, N.Y.), and HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, VOLUMES I-IV (D.M. Weir and C. C. Blackwell eds 1986).

[0115] All patents, patent applications, and publications mentioned herein, both supra and infra, are hereby incorporated herein by reference.

[0116] The useful materials and processes of the present invention are made possible by the provision of a family of closely homologous nucleotide sequences isolated from a cDNA library derived from nucleic acid sequences present in the plasma of an HCV infected chimpanzee. This family of nucleotide sequences is not of human or chimpanzee origin, since it hybridizes to neither human nor chimpanzee genomic DNA from uninfected individuals, since nucleotides of this family of sequences are present only in liver and plasma of chimpanzees with HCV infection, and since the sequence is not present in Genbank. In addition, the family of sequences shows no significant homology to sequences contained within the HBV genome.

[0117] The sequence of one member of the family, contained within clone 5-1-1, has one continuous open reading frame (ORF) which encodes a polypeptide of approximately 50 amino acids. Sera from HCV infected humans contain antibodies which bind to this polypeptide, whereas sera from non-infected humans do not contain antibodies to this polypeptide. Finally, whereas the sera from uninfected chimpanzees do not contain antibodies to this polypeptide, the antibodies are induced in chimpanzees following acute NANBH infection. Moreover, antibodies to this polypeptide are not detected in chimps and humans infected with HAV and HBV. By these criteria the sequence is a cDNA to a viral sequence, wherein the virus causes or is associated with NANBH; this cDNA sequence is shown in Fig. 1. As discussed infra, the cDNA sequence in clone 5-1-1 differs from that of the other isolated cDNAs in that it contains 28 extra base

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pairs.

[0118] A composite of other identified members of the cDNA family, which were isolated using as a probe a synthetic sequence equivalent to a fragment of the cDNA in clone 5-1-1, is shown in Fig. 3. A member of the cDNA family which was isolated using a synthetic sequence derived from the cDNA in clone 81 is shown in Fig. 5, and the composite of this sequence with that of clone 81 is shown in Fig. 6. Other members of the cDNA family, including those present in clones 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g and 15e are described in Section IV.A. A composite of the cDNAs in these clones is described in Section IV.A. 19, and shown in Fig. 32. The composite cDNA shows that it contains one continuous ORF, and thus encodes a polyprotein. This data is consistent with the suggestion, discussed infra., that HCV is a flavivirus or flavi-like virus. Clone k9-1 overlaps the sequence of Fig. 32. A composite cDNA is shown in Fig. 47.

[0119] The availability of this family of cDNAs shown in Figs. 1-47, inclusive, permits the construction of DNA probes and polypeptides useful in diagnosing NANBH due to HCV infection and in screening blood donors as well as donated blood and blood products for infection. For example, from the sequences it is possible to synthesize DNA oligomers of about 8-10 nucleotides, or larger, which are useful as hybridization probes to detect the presence of the viral genome in, for example, sera of subjects suspected of harboring the virus, or for screening donated blood for the presence of the virus. The family of cDNA sequences also allows the design and production of HCV specific polypeptides which are useful as diagnostic reagents for the presence of antibodies raised during NANBH. Antibodies to purified polypeptides derived from the cDNAs may also be used to detect viral antigens in infected individuals and in blood.

[0120] Knowledge of these cDNA sequences also enable the design and production of polypeptides which may be used as vaccines against HCV and also for the production of antibodies, which in turn may be used for protection against the disease, and/or for therapy of HCV infected individuals.

[0121] Moreover, the family of cDNA sequences enables further characterization of the HCV genome. Polynucleotide probes derived from these sequences may be used to screen cDNA libraries for additional overlapping cDNA sequences, which, in turn, may be used to obtain more overlapping sequences. Unless the genome is segmented and the segments lack common sequences, this technique may be used to gain the sequence of the entire genome. However, if the genome is segmented, other segments of the genome can be obtained by repeating the lambda-gt11 serological screening procedure used to isolate the cDNA clones described herein, or alternatively by isolating the genome from purified HCV particles.

[0122] The family of cDNA sequences and the polypeptides derived from these sequences, as well as antibodies directed against these polypeptides are also useful in the isolation and identification of the BB-NANBV agent(s). For example, antibodies directed against HCV epitopes contained in polypeptides derived from the cDNAs may be used in processes based upon affinity chromatography to isolate the virus. Alternatively, the antibodies may be used to identify viral particles isolated by other techniques. The viral antigens and the genomic material within the isolated viral particles may then be further characterized.

[0123] The information obtained from further sequencing of the HCV genome(s), as well as from further characterization of the HCV antigens and characterization of the genome enables the design and synthesis of additional probes and polypeptides and antibodies which may be used for diagnosis, for prevention, and for therapy of HCV induced NANBH, and for screening for infected blood and blood-related products.

[0124] The availability of probes for HCV, including antigens and antibodies, and polynucleotides derived from the genome from which the family of cDNAs is derived also allows for the development of tissue culture systems which will be of major use in elucidating the biology of HCV. This in turn, may lead to the development of new treatment regimens based upon antiviral compounds which preferentially inhibit the replication of, or infection by HCV.

[0125] The method used to identify and isolate the etiologic agent for NANBH is novel, and it may be applicable to the identification and/or isolation of heretofore uncharacterized agents which contain a genome, and which are associated with a variety of diseases, including those induced by viruses, viroids, bacteria, fungi and parasites. In this method, a cDNA library was created from the nucleic acids present in infected tissue from an infected individual. The library was created in a vector which allowed the expression of polypeptides encoded in the cDNA. Clones of host cells containing the vector, which expressed an immunologically reactive fragment of a polypeptide of the etiologic agent, were selected by immunological screening of the expression products of the library with an antibody containing body component from another individual previously infected with the putative agent. The steps in the immunological screening technique included interacting the expression products of the cDNA containing vectors with the antibody containing body component of a second infected individual, and detecting the formation of antibody-antigen complexes between the expression product(s) and antibodies of the second infected individual. The isolated clones are screened further immunologically by interacting their expression products with the antibody containing body components of other individuals infected with the putative agent and with control individuals uninfected with the putative agent, and detecting the formation of antigen-antibody complexes with antibodies from the infected individuals; and the cDNA containing vectors which encode polypeptides which react immunologically with antibodies from infected individuals and individuals suspected of being infected with the agent, but not with control individuals are isolated. The infected individuals

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used for the construction of the cDNA library, and for the immunological screening need not be of the same species.

[0126] The cDNAs isolated as a result of this method, and their expression products, and antibodies directed against the expression products, are useful in characterizing and/or capturing the etiologic agent. As described in more detail infra, this method has been used successfully to isolate a family of cDNAs derived from the HCV genome.

## II A. Preparation of the cDNA Sequence

[0127] Pooled serum from a chimpanzee with chronic HCV infection and containing a high titer of the virus, i.e., at least  $10^6$  chimp infectious doses/ml (CID/ml) was used to isolate viral particles; nucleic acids isolated from these particles was used as the template in the construction of a cDNA library to the viral genome. The procedures for isolation of putative HCV particles and for constructing the cDNA library in lambda-gt11 is discussed in Section IV.A.1. Lambda-gt11 is a vector that has been developed specifically to express inserted cDNAs as fusion polypeptides with beta-galactosidase and to screen large numbers of recombinant phage with specific antisera raised against a defined antigen. The lambda-gt11 cDNA library generated from a cDNA pool containing cDNA of approximate mean size of 200 base pairs was screened for encoded epitopes that could bind specifically with sera derived from patients who had previously experienced NANB hepatitis. Huynh, T.V. et al. (1985). Approximately  $10^6$  phages were screened, and five positive phages were identified, purified, and then tested for specificity of binding to sera from different humans and chimpanzees previously infected with the HCV agent. One of the phages, 5-1-1, bound 5 of the 8 human sera tested. This binding appeared selective for sera derived from patients with prior NANB hepatitis infections since 7 normal blood donor sera did not exhibit such binding.

[0128] The sequence of the cDNA in recombinant phage 5-1-1 was determined, and is shown in Fig. 1. The polypeptide encoded by this cloned cDNA, which is in the same translational frame as the N-terminal beta-Galactosidase moiety of the fusion polypeptide is shown above the nucleotide sequence. This translational ORF, therefore, encodes an epitope(s) specifically recognized by sera from patients with NANB hepatitis infections.

[0129] The availability of the cDNA in recombinant phage 5-1-1 has allowed for the isolation of other clones containing additional segments and/or alternative segments of cDNA to the viral genome. The lambda-gt11 cDNA library described supra, was screened using a synthetic polynucleotide derived from the sequence of the cloned 5-1-1 cDNA. This screening yielded three other clones, which were identified as 81, 1-2 and 91; the cDNAs contained within these clones were sequenced. See Sections IV.A.3. and IV.A.4. The homologies between the four independent clones are shown in Fig. 2, where the homologies are indicated by the vertical lines. Sequences of nucleotides present uniquely in clones 5-1-1, 81, and 91 are indicated by small letters.

[0130] The cloned cDNAs present in recombinant phages in clones 5-1-1, 81, 1-2, and 91 are highly homologous, and differ in only two regions. First, nucleotide number 67 in clone 1-2 is a thymidine, whereas the other three clones contain a cytidine residue in this position. This substitution, however, does not alter the nature of the encoded amino acid.

[0131] The second difference between the clones is that clone 5-1-1 contains 28 base pairs at its 5'-terminus which are not present in the other clones. The extra sequence may be a 5'-terminal cloning artifact; 5'-terminal cloning artifacts are commonly observed in the products of cDNA methods.

[0132] Synthetic sequences derived from the 5'-region and the 3'-region of the HCV cDNA in clone 81 were used to screen and isolate cDNAs from the lambda-gt11 NANBV cDNA library, which overlapped clone 81 cDNA (Section IV.A.5.). The sequences of the resulting cDNAs, which are in clone 36 and clone 32, respectively, are shown in Fig. 5 and Fig. 7.

[0133] Similarly, a synthetic polynucleotide based on the 5'-region of clone 36 was used to screen and isolate cDNAs from the lambda-gt11 NANBV cDNA library which overlapped clone 36 cDNA (Section IV.A.8.). A purified clone of recombinant phage-containing cDNA which hybridized to the synthetic polynucleotide probe was named clone 35 and the NANBV cDNA sequence contained within this clone is shown in Fig. 8.

[0134] By utilizing the technique of isolating overlapping cDNA sequences, clones containing additional upstream and downstream HCV cDNA sequences have been obtained. The isolation of these clones, is described infra in Section IV.A.

[0135] Analysis of the nucleotide sequences of the HCV cDNAs encoded within the isolated clones show that the composite cDNA contains one long continuous ORF. Fig. 26 shows the sequence of the composite cDNA from these clones, along with the putative HCV polypeptide encoded therein.

[0136] The description of the method to retrieve the cDNA sequences is mostly of historical interest. The resultant sequences (and their complements) are provided herein, and the sequences, or any portion thereof, could be prepared using synthetic methods, or by a combination of synthetic methods with retrieval of partial sequences using methods similar to those described herein.

[0137] Lambda-gt11 strains replicated from the HCV cDNA library and from clones 5-1-1, 81, 1-2 and 91 have been deposited under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn

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Dr., Rockville, Maryland 20852, and have been assigned the following Accession Numbers.

lambda-gt11	ATCC No.	Deposit Date
HCV cDNA library	40394	1 Dec. 1987
clone 81	40388	17 Nov. 1987
clone 91	40389	17 Nov. 1987
clone 1-2	40390	17 Nov. 1987
clone 5-1-1	40391	18 Nov. 1987

[0138] The designated deposits will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit; or for the enforceable life of the U.S. patent, whichever is longer. These deposits and other deposited materials mentioned herein are intended for convenience only, and are not required to practice the present invention in view of the description here. The HCV cDNA sequences in all of the deposited materials are incorporated herein by reference.

[0139] The description above, of "walking" the genome by isolating overlapping cDNA sequences from the HCV lambda gt-11 library provides one method by which cDNAs corresponding to the entire HCV genome may be isolated. However, given the information provided herein, other methods for isolating these cDNAs are obvious to one of skill in the art. Some of these methods are described in Section IV.A., *infra*.

#### II.B. Preparation of Viral Polypeptides and Fragments

[0140] The availability of cDNA sequences, either those isolated by utilizing the cDNA sequences in Figs. 1-32, as discussed *infra*, as well as the cDNA sequences in these figures, permits the construction of expression vectors encoding antigenically active regions of the polypeptide encoded in either strand. These antigenically active regions may be derived from coat or envelope antigens or from core antigens, including, for example, polynucleotide binding proteins, polynucleotide polymerase(s), and other viral proteins required for the replication and/or assembly of the virus particle. Fragments encoding the desired polypeptides are derived from the cDNA clones using conventional restriction digestion or by synthetic methods, and are ligated into vectors which may, for example, contain portions of fusion sequences such as beta-Galactosidase or superoxide dismutase (SOD), preferably SOD. Methods and vectors which are useful for the production of polypeptides which contain fusion sequences of SOD are described in European Patent Office Publication number 0196056, published October 1, 1986. Vectors encoding fusion polypeptides of SOD and HCV polypeptides, i.e., NANB<sub>5-1-1</sub>, NAN<sub>81</sub>, and C100-3, which is encoded in a composite of HCV cDNAs, are described in Sections IV.B.1, IV.B.2, and IV.B.4, respectively. Any desired portion of the HCV cDNA containing an open reading frame, in either sense strand, can be obtained as a recombinant polypeptide, such as a mature or fusion protein; alternatively, a polypeptide encoded in the cDNA can be provided by chemical synthesis.

[0141] The DNA encoding the desired polypeptide, whether in fused or mature form, and whether or not containing a signal sequence to permit secretion, may be ligated into expression vectors suitable for any convenient host. Both eukaryotic and prokaryotic host systems are presently used in forming recombinant polypeptides, and a summary of some of the more common control systems and host cell lines is given in Section III.A., *infra*. The polypeptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification may be by techniques known in the art, for example, salt fractionation, chromatography on ion exchange resins, affinity chromatography, centrifugation, and the like. See, for example, Methods in Enzymology for a variety of methods for purifying proteins. Such polypeptides can be used as diagnostics, or those which give rise to neutralizing antibodies may be formulated into vaccines. Antibodies raised against these polypeptides can also be used as diagnostics, or for passive immunotherapy. In addition, as discussed in Section II.J. herein below, antibodies to these polypeptides are useful for isolating and identifying HCV particles.

[0142] The HCV antigens may also be isolated from HCV virions. The virions may be grown in HCV infected cells in tissue culture, or in an infected host.

#### II.C. Preparation of Antigenic Polypeptides and Conjugation with Carrier

[0143] An antigenic region of a polypeptide is generally relatively small—typically 8 to 10 amino acids or less in length. Fragments of as few as 5 amino acids may characterize an antigenic region. These segments may correspond to regions of HCV antigen. Accordingly, using the cDNAs of HCV as a basis, DNAs encoding short segments of HCV polypeptides can be expressed recombinantly either as fusion proteins, or as isolated polypeptides. In addition, short amino acid sequences can be conveniently obtained by chemical synthesis. In instances wherein the synthesized

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polypeptide is correctly configured so as to provide the correct epitope, but is too small to be immunogenic, the polypeptide may be linked to a suitable carrier.

[0144] A number of techniques for obtaining such linkage are known in the art, including the formation of disulfide linkages using N-succinimidyl-3-(2-pyridylthio)propionate (SPDP) and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) obtained from Pierce Company, Rockford, Illinois, (if the peptide lacks a sulfhydryl group, this can be provided by addition of a cysteine residue.) These reagents create a disulfide linkage between themselves and peptide cysteine residues on one protein and an amide linkage through the epsilon-amino group on a lysine, or other free amino group in the other. A variety of such disulfide/amide-forming agents are known. See, for example, Immun. Rev. (1982) 62:185. Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thioether-forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic acid, 2-iodoacetic acid, 4-(N-maleimidomethyl)cyclohexane-1-carboxylic acid, and the like. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxyl-2-nitro-4-sulfonic acid, sodium salt. The foregoing list is not meant to be exhaustive, and modifications of the named compounds can clearly be used.

[0145] Any carrier may be used which does not itself induce the production of antibodies harmful to the host. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins; polysaccharides, such as latex functionalized sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids, such as polyglutamic acid, polylysine, and the like; amino acid copolymers; and inactive virus particles, see, for example, section II.D. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those skilled in the art.

#### II.D. Preparation of Hybrid Particle Immunogens Containing HCV Epitopes

[0146] The immunogenicity of the epitopes of HCV may also be enhanced by preparing them in mammalian or yeast systems fused with or assembled with particle-forming proteins such as, for example, that associated with hepatitis B surface antigen. Constructs wherein the NANBV epitope is linked directly to the particle-forming protein coding sequences produce hybrids which are immunogenic with respect to the HCV epitope. In addition, all of the vectors prepared include epitopes specific to HBV, having various degrees of immunogenicity, such as, for example, the pre-S peptide. Thus, particles constructed from particle forming protein which include HCV sequences are immunogenic with respect to HCV and HBV.

[0147] Hepatitis surface antigen (HBsAg) has been shown to be formed and assembled into particles in *S. cerevisiae* (Valenzuela et al. (1982)), as well as in, for example, mammalian cells (Valenzuela, P., et al. (1984)). The formation of such particles has been shown to enhance the immunogenicity of the monomer subunit. The constructs may also include the immunodominant epitope of HBsAg, comprising the 55 amino acids of the presurface (pre-S) region. Neurath et al. (1984). Constructs of the pre-S-HBsAg particle expressible in yeast are disclosed in EPO 174,444, published March 19, 1986; hybrids including heterologous viral sequences for yeast expression are disclosed in EPO 175,261, published March 26, 1986. Both applications are assigned to the herein assignee, and are incorporated herein by reference. These constructs may also be expressed in mammalian cells such as Chinese hamster ovary (CHO) cells using an SV40-dihydrofolate reductase vector (Michelle et al. (1984)).

[0148] In addition, portions of the particle-forming protein coding sequence may be replaced with codons encoding an HCV epitope. In this replacement, regions which are not required to mediate the aggregation of the units to form immunogenic particles in yeast or mammals can be deleted, thus eliminating additional HBV antigenic sites from competition with the HCV epitope.

#### II.E. Preparation of Vaccines

[0149] Vaccines may be prepared from one or more immunogenic polypeptides derived from HCV cDNA as well as from the cDNA sequences in the Figs. 1-32, or from the HCV genome to which they correspond. The observed homology between HCV and Flaviviruses provides information concerning the polypeptides which are likely to be most effective as vaccines, as well as the regions of the genome in which they are encoded. The general structure of the Flavivirus genome is discussed in Rice et al (1986). The flavivirus genomic RNA is believed to be the only virus-specific mRNA species, and it is translated into the three viral structural proteins, i.e., C, M, and E, as well as two large nonstructural proteins, NV4 and NV5, and a complex set of smaller nonstructural proteins. It is known that major neutralizing epitopes for Flaviviruses reside in the E (envelope) protein (Roehrig (1986)). The corresponding HCV E gene and polypeptide encoding region can be predicted, based upon the homology to Flaviviruses. Thus, vaccines may be comprised of recombinant polypeptides containing epitopes of HCV E. These polypeptides may be expressed in bacteria, yeast, or mammalian cells, or alternatively may be isolated from viral preparations. It is also anticipated that the other structural proteins may also contain epitopes which give rise to protective anti-HCV antibodies. Thus, polypeptides containing the epitopes of E, C, and M may also be used, whether singly or in combination, in HCV vaccines.

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[0150] In addition to the above, it has been shown that immunization with NS1 (nonstructural protein 1), results in protection against yellow fever (Schlesinger et al (1986)). This is true even though the immunization does not give rise to neutralizing antibodies. Thus, particularly since this protein appears to be highly conserved among Flaviviruses, it is likely that HCV NS1 will also be protective against HCV infection. Moreover, it also shows that nonstructural proteins may provide protection against viral pathogenicity, even if they do not cause the production of neutralizing antibodies.

[0151] In view of the above, multivalent vaccines against HCV may be comprised of one or more structural proteins, and/or one or more nonstructural proteins. These vaccines may be comprised of, for example, recombinant HCV polypeptides and/or polypeptides isolated from the virions. In addition, it may be possible to use inactivated HCV in vaccines; inactivation may be by the preparation of viral lysates, or by other means known in the art to cause inactivation of Flaviviruses, for example, treatment with organic solvents or detergents, or treatment with formalin. Moreover, vaccines may also be prepared from attenuated HCV strains. The preparation of attenuated HCV strains is described infra.

[0152] It is known that some of the proteins in Flaviviruses contain highly conserved regions, thus, some immunological cross-reactivity is expected between HCV and other Flaviviruses. It is possible that shared epitopes between the Flaviviruses and HCV will give rise to protective antibodies against one or more of the disorders caused by these pathogenic agents. Thus, it may be possible to design multipurpose vaccines based upon this knowledge.

[0153] The preparation of vaccines which contain an immunogenic polypeptide(s) as active ingredients, is known to one skilled in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the protein encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonine-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against an immunogenic polypeptide containing an HCV antigenic sequence resulting from administration of this polypeptide in vaccines which are also comprised of the various adjuvants.

[0154] The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25%-70%.

[0155] The proteins may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

**II.F. Dosage and Administration of Vaccines**

[0156] The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of 5 micrograms to 250 micrograms of antigen per dose, depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend on the judgment of the practitioner and may be peculiar to each subject.

[0157] The vaccine may be given in a single dose schedule, or preferably in a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and/or reinforce the immune response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of the individual and be dependent upon the judgment of the practitioner.

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[0158] In addition, the vaccine containing the immunogenic HCV antigen(s) may be administered in conjunction with other immunoregulatory agents, for example, immune globulins.

## II.G. Preparation of Antibodies Against HCV Epitopes

[0159] The immunogenic polypeptides prepared as described above are used to produce antibodies, both polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunized with an immunogenic polypeptide bearing an HCV epitope(s). Serum from the immunized animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to an HCV epitope contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art, see for example, Mayer and Walker (1987).

[0160] Alternatively, polyclonal antibodies may be isolated from a mammal which has been previously infected with HCV. An example of a method for purifying antibodies to HCV epitopes from serum from an infected individual, based upon affinity chromatography and utilizing a fusion polypeptide of SOD and a polypeptide encoded within cDNA clone 5-1-1, is presented in Section V.E.

[0161] Monoclonal antibodies directed against HCV epitopes can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al. (1980); Hammerling et al. (1981); Kennett et al. (1980); see also, U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,466,917; 4,472,500; 4,491,632; and 4,493,890. Panels of monoclonal antibodies produced against HCV epitopes can be screened for various properties; i.e., for isotype, epitope affinity, etc.

[0162] Antibodies, both monoclonal and polyclonal, which are directed against HCV epitopes are particularly useful in diagnosis, and those which are neutralizing are useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotypic antibodies.

[0163] Anti-idiotypic antibodies are immunoglobulins which carry an "internal image" of the antigen of the infectious agent against which protection is desired. See, for example, Nisonoff, A., et al. (1981) and Dreesman et al. (1985).

[0164] Techniques for raising anti-idiotypic antibodies are known in the art. See, for example, Grzych (1985), MacNamara et al. (1984), and Uytendaele et al. (1985). These anti-idiotypic antibodies may also be useful for treatment of NANBH, as well as for an elucidation of the immunogenic regions of HCV antigens.

## II.H. Diagnostic Oligonucleotide Probes and Kits

[0165] Using the disclosed portions of the isolated HCV cDNAs as a basis, including those in Figs. 1-32, oligomers of approximately 8 nucleotides or more can be prepared, either by excision or synthetically, which hybridize with the HCV genome and are useful in identification of the viral agent(s), further characterization of the viral genome(s), as well as in detection of the virus(es) in diseased individuals. The probes for HCV polynucleotides (natural or derived) are a length which allows the detection of unique viral sequences by hybridization. While 6-8 nucleotides may be a workable length, sequences of 10-12 nucleotides are preferred, and about 20 nucleotides appears optimal. Preferably, these sequences will derive from regions which lack heterogeneity. These probes can be prepared using routine methods, including automated oligonucleotide synthetic methods. Among useful probes, for example, are the clone 5-1-1 and the additional clones disclosed herein, as well as the various oligomers useful in probing cDNA libraries, set forth below. A complement to any unique portion of the HCV genome will be satisfactory. For use as probes, complete complementarity is desirable, though it may be unnecessary as the length of the fragment is increased.

[0166] For use of such probes as diagnostics, the biological sample to be analyzed, such as blood or serum, is treated, if desired, to extract the nucleic acids contained therein. The resulting nucleic acid from the sample may be subjected to gel electrophoresis or other size separation techniques; alternatively, the nucleic acid sample may be dot blotted without size separation. The probes are then labeled. Suitable labels, and methods for labeling probes are known in the art, and include, for example, radioactive labels incorporated by nick translation or kinasing, biotin, fluorescent probes, and chemiluminescent probes. The nucleic acids extracted from the sample are then treated with the labeled probe under hybridization conditions of suitable stringencies.

[0167] The probes can be made completely complementary to the HCV genome. Therefore, usually high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency should only be used if the probes are complementary to regions of the viral genome which lack heterogeneity. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time, and concentration of formamide. These factors are outlined in, for example, Maniatis, T. (1982).

[0168] Generally, it is expected that the HCV genome sequences will be present in serum of infected individuals at

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relatively low levels, i.e., at approximately  $10^2$ - $10^3$  sequences per ml. This level may require that amplification techniques be used in hybridization assays. Such techniques are known in the art. For example, the Enzo Biochemical Corporation "Bio-Bridge" system uses terminal deoxynucleotide transferase to add unmodified 3'-poly-dT-tails to a DNA probe. The poly dT-tailed probe is hybridized to the target nucleotide sequence, and then to a biotin-modified poly-A. PCT application 84/03520 and EPA124221 describe a DNA hybridization assay in which: (1) analyte is annealed to a single-stranded DNA probe that is complementary to an enzyme-labeled oligonucleotide; and (2) the resulting

5   tailed duplex is hybridized to an enzyme-labeled oligonucleotide. EPA 204510 describes a DNA hybridization assay in which analyte DNA is contacted with a probe that has a tail, such as a poly-dT tail, an amplifier strand that has a sequence that hybridizes to the tail of the probe, such as a poly-A sequence, and which is capable of binding a plurality

10   of labeled strands. A particularly desirable technique may first involve amplification of the target HCV sequences in sera approximately 10,000 fold, i.e., to approximately  $10^6$  sequences/ml. This may be accomplished, for example, by the technique of Saiki et al. (1986). The amplified sequence(s) may then be detected using a hybridization assay. A suitable solution phase sandwich assay which may be used with labeled polynucleotide probes, and the methods for the preparation of probes is described in EPO 225,807, published June 16, 1987, which is assigned to the herein

15   assignee, and which is hereby incorporated herein by reference.

[0169] The probes can be packaged into diagnostic kits. Diagnostic kits include the probe DNA, which may be labeled; alternatively, the probe DNA may be unlabeled and the ingredients for labeling may be included in the kit. The kit may also contain other suitably packaged reagents and materials needed for the particular hybridization protocol, for example, standards, as well as instructions for conducting the test.

20

#### II-I. Immunoassay and Diagnostic Kits

[0170] Both the polypeptides which react immunologically with serum containing HCV antibodies, for example, those derived from or encoded within the clones described in Section IV.A., and composites thereof, (see section IV.A.) and the antibodies raised against the HCV specific epitopes in these polypeptides, see for example Section IV.E, are useful

25   in immunoassays to detect presence of HCV antibodies, or the presence of the virus and/or viral antigens, in biological samples, including for example, blood or serum samples. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. For example, the immunoassay may utilize one viral antigen, for example, a polypeptide derived from any of the clones containing HCV cDNA described in Section IV.A., or from the composite cDNAs derived from the cDNAs in these clones, or from the HCV genome from which the cDNA in these

30   clones is derived; alternatively, the immunoassay may use a combination of viral antigens derived from these sources. It may use, for example, a monoclonal antibody directed towards a viral epitope(s), a combination of monoclonal antibodies directed towards one viral antigen, monoclonal antibodies directed towards different viral antigens, polyclonal antibodies directed towards the same viral antigen, or polyclonal antibodies directed towards different viral antigens.

35   Protocols may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

[0171] The Flavivirus model for HCV allows predictions regarding the likely location of diagnostic epitopes for the virion structural proteins. The C, pre-M, M, and E domains are all likely to contain epitopes of significant potential for detecting viral antigens, and particularly for diagnosis. Similarly, domains of the nonstructural proteins are expected to contain important diagnostic epitopes (e.g., NS5 encoding a putative polymerase; and NS1 encoding a putative complement-binding antigen). Recombinant polypeptides, or viral polypeptides, which include epitopes from these

45   specific domains may be useful for the detection of viral antibodies in infections blood donors and infected patients.

[0172] In addition, antibodies directed against the E and/or M proteins can be used in immunoassays for the detection of viral antigens in patients with HCV caused NANBH, and in infectious blood donors. Moreover, these antibodies will be extremely useful in detecting acute-phase donors and patients.

[0173] Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the polypeptides of the invention containing HCV epitopes or antibodies directed against HCV epitopes in suitable containers, along with the remaining reagents and materials required for the conduct of the assay, as well as a suitable set of assay instructions.

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#### II.J. Further Characterization of the HCV Genome, Virions, and Viral Antigens Using Probes Derived From cDNA to the Viral Genome

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[0174] The HCV cDNA sequence information in the clones described in Section IV.A., as shown in Figs. 1-32, inclusive, may be used to gain further information on the sequence of the HCV genome, and for identification and isolation

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of the HCV agent, and thus will aid in its characterization including the nature of the genome, the structure of the viral particle, and the nature of the antigens of which it is composed. This information, in turn, can lead to additional polynucleotide probes, polypeptides derived from the HCV genome, and antibodies directed against HCV epitopes which would be useful for the diagnosis and/or treatment of HCV caused NANBH.

5 **[0175]** The cDNA sequence information in the above-mentioned clones is useful for the design of probes for the isolation of additional cDNA sequences which are derived from as yet undefined regions of the HCV genome(s) from which the cDNAs in clones described in Section IV.A. are derived. For example, labeled probes containing a sequence of approximately 8 or more nucleotides, and preferably 20 or more nucleotides, which are derived from regions close to the 5'-termini or 3'-termini of the family of HCV cDNA sequences shown in Figs. 1, 3, 6, 9, 14 and 32 may be used to isolate overlapping cDNA sequences from HCV cDNA libraries. These sequences which overlap the cDNAs in the above-mentioned clones, but which also contain sequences derived from regions of the genome from which the cDNA in the above mentioned clones are not derived, may then be used to synthesize probes for identification of other overlapping fragments which do not necessarily overlap the cDNAs in the clones described in Section IV.A. Unless the HCV genome is segmented and the segments lack common sequences, it is possible to sequence the entire viral genome(s) utilizing the technique of isolation of overlapping cDNAs derived from the viral genome(s). Although it is unlikely, if the genome is a segmented genome which lacks common sequences, the sequence of the genome can be determined by serologically screening lambda-gt11 HCV cDNA libraries, as used to isolate clone 5-1-1, sequencing cDNA isolates, and using the isolated cDNAs to isolate overlapping fragments, using the technique described for the isolation and sequencing of the clones described in Section IV.A. Alternatively, characterization of the genomic segments could be from the viral genome(s) isolated from purified HCV particles. Methods for purifying HCV particles and for detecting them during the purification procedure are described herein, *infra*. Procedures for isolating polynucleotide genomes from viral particles are, known in the art, and one procedure which may be used is shown in Example IV.A. 1. The isolated genomic segments could then be cloned and sequenced. Thus, with the information provided herein, it is possible to clone and sequence the HCV genome(s) irrespective of their nature.

25 **[0176]** Methods for constructing cDNA libraries are known in the art, and are discussed *supra* and *infra*; a method for the construction of HCV cDNA libraries in lambda-gt11 is discussed *infra* in Section IV.A. However, cDNA libraries which are useful for screening with nucleic acid probes may also be constructed in other vectors known in the art, for example, lambda-gt10 (Huynh et al. (1985)). The HCV derived cDNA detected by the probes derived from the cDNAs in Figs. 1-32, and from the probes synthesized from polynucleotides derived from these cDNAs, may be isolated from the clone by digestion of the isolated polynucleotide with the appropriate restriction enzyme(s), and sequenced. See, for example, Section IV.A.3. and IV.A.4. for the techniques used for the isolation and sequencing of HCV cDNA which overlaps HCV cDNA in clone 5-1-1, Sections IV.A.5-IV.A.7 for the isolation and sequencing of HCV cDNA which overlaps that in clone 81, and Section IV.A.8 and IV.A.9 for the isolation and sequencing of a clone which overlaps another clone (clone 36), which overlaps clone 81.

35 **[0177]** The sequence information derived from these overlapping HCV cDNAs is useful for determining areas of homology and heterogeneity within the viral genome(s), which could indicate the presence of different strains of the genome, and/or of populations of defective particles. It is also useful for the design of hybridization probes to detect HCV or HCV antigens or HCV nucleic acids in biological samples, and during the isolation of HCV (discussed *infra*), utilizing the techniques described in Section II.G. Moreover, the overlapping cDNAs may be used to create expression vectors for polypeptides derived from the HCV genome(s) which also encode the polypeptides encoded in clones 5-1-1, 36, 81, 91, and 1-2, and in the other clones described in Section IV.A. The techniques for the creation of these polypeptides containing HCV epitopes, and for antibodies directed against HCV epitopes contained within them, as well as their uses, are analogous to those described for polypeptides derived from NANBV cDNA sequences contained within clones 5-1-1, 32, 35, 36, 1-2, 81, and 91, discussed *supra* and *infra*.

45 **[0178]** Encoded within the family of cDNA sequences contained within clones 5-1-1, 32, 35, 36, 81, 91, 1-2, and the other clones described in Section IV.A. are antigen(s) containing epitopes which appear to be unique to HCV; i.e., antibodies directed against these antigens are absent from individuals infected with HAV or HBV, and from individuals not infected with HCV (see the serological data presented in Section IV.B.). Moreover, a comparison of the sequence information of these cDNAs with the sequences of HAV, HBV, HDV, and with the genomic sequences in Genbank indicates that minimal homology exists between these cDNAs and the polynucleotide sequences of those sources. Thus, antibodies directed against the antigens encoded within the cDNAs of these clones may be used to identify BB-NANBV particles isolated from infected individuals. In addition, they are also useful for the isolation of NANBH agent(s).

55 **[0179]** HCV particles may be isolated from the sera from BB-NANBV infected individuals or from cell cultures by any of the methods known in the art, including for example, techniques based on size discrimination such as sedimentation or exclusion methods, or techniques based on density such as ultracentrifugation in density gradients, or precipitation with agents such as polyethylene glycol, or chromatography on a variety of materials such as anionic or cationic exchange materials, and materials which bind due to hydrophobicity, as well as affinity columns. During the isolation procedure the presence of HCV may be detected by hybridization analysis of the extracted genome, using probes

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derived from the HCV cDNAs described supra, or by immunoassay (see Section II.I.) utilizing as probes antibodies directed against HCV antigens encoded within the family of cDNA sequences shown in Figs. 1-32, and also directed against HCV antigens encoded within the overlapping HCV cDNA sequences discussed supra. The antibodies may be monoclonal, or polyclonal, and it may be desirable to purify the antibodies before their use in the immunoassay. A purification procedure for polyclonal antibodies directed against antigen(s) encoded within clone 5-1-1 is described in Section IV.E; analogous purification procedures may be utilized for antibodies directed against other HCV antigens.

**[0180]** Antibodies directed against HCV antigens encoded within the family of cDNAs shown in Figs. 1-32, as well as those encoded within overlapping HCV cDNAs, which are affixed to solid supports are useful for the isolation of HCV by immunoaffinity chromatography. Techniques for immunoaffinity chromatography are known in the art, including techniques for affixing antibodies to solid supports so that they retain their immunoselective activity; the techniques may be those in which the antibodies are adsorbed to the support (see, for example, Kurstak in *ENZYME IMMUNO-DIAGNOSIS*, page 31-37), as well as those in which the antibodies are covalently linked to the support. Generally, the techniques are similar to those used for covalent linking of antigens to a solid support, which are generally described in Section II.C.; however, spacer groups may be included in the bifunctional coupling agents so that the antigen binding site of the antibody remains accessible.

**[0181]** During the purification procedure the presence of HCV may be detected and/or verified by nucleic acid hybridization, utilizing as probes polynucleotides derived from the family of HCV cDNA sequences shown in Figs. 1-32, as well as from overlapping HCV cDNA sequences, described supra. In this case, the fractions are treated under conditions which would cause the disruption of viral particles, for example, with detergents in the presence of chelating agents, and the presence of viral nucleic acid determined by hybridization techniques described in Section II.H. Further confirmation that the isolated particles are the agents which induce HCV may be obtained by infecting chimpanzees with the isolated virus particles, followed by a determination of whether the symptoms of NANBH result from the infection.

**[0182]** Viral particles from the purified preparations may then be further characterized. The genomic nucleic acid has been purified. Based upon its sensitivity to RNase, and not DNase I, it appears that the virus is composed of an RNA genome. See Example IV.C.2., *infra*. The strandedness and circularity or non-circularity can be determined by techniques known in the art, including, for example, its visualization by electron microscopy, its migration in density gradients, and its sedimentation characteristics. Based upon the hybridization of the captured HCV genome to the negative strands of HCV cDNAs, it appears that HCV may be comprised of a positive stranded RNA genome (see Section IV.H.1). Techniques such as these are described in, for example, *METHODS IN ENZYMOLOGY*. In addition, the purified nucleic acid can be cloned and sequenced by known techniques, including reverse transcription since the genomic material is RNA. See, for example, Maniatis (1982), and Glover (1985). Utilizing the nucleic acid derived from the viral particles, it is possible to sequence the entire genome, whether or not it is segmented.

**[0183]** Examination of the homology of the polypeptide encoded within the continuous ORF of combined clones 14i through 39c (see Fig. 26), shows that the HCV polypeptide contains regions of homology with the corresponding proteins in conserved regions of flaviviruses. An example of this is described in Section IV.H.3. This finding has many important ramifications. First, this evidence, in conjunction with the results which show that HCV contains a positive-stranded genome, the size of which is approximately 10,000 nucleotides, is consistent with the suggestion that HCV is a flavivirus, or flavi-like virus. Generally, flavivirus virions and their genomes have a relatively consistent structure and organization, which are known. See Rice et al. (1986), and Brinton, M.A. (1988). Thus, the structural genes encoding the polypeptides C, pre-M/M, and E may be located in the 5'-terminus of the genome upstream of clone 14i. Moreover, using the comparison with other flaviviruses, predictions as to the precise location of the sequences encoding these proteins can be made.

**[0184]** Isolation of the sequences upstream of those in clone 14i may be accomplished in a number of ways which, given the information herein, would be obvious to one of skill in the art. For example, the genome "walking" technique, may be used to isolate other sequences which are 5' to those in clone 14i, but which overlap that clone; this in turn leads to the isolation of additional sequences. This technique has been amply demonstrated *infra*, in Section IV.A.. For example, also, it is known that the flaviviruses have conserved epitopes and regions of conserved nucleic acid sequences. Polynucleotides containing the conserved sequences may be used as probes which bind the HCV genome, thus allowing its isolation. In addition, these conserved sequences, in conjunction with those derived from the HCV cDNAs shown in Fig. 22, may be used to design primers for use in systems which amplify the genome sequences upstream of those in clone 14i, using polymerase chain reaction technology. An example of this is described *infra*.

**[0185]** The structure of the HCV may also be determined and its components isolated. The morphology and size may be determined by, for example, electron microscopy. The identification and localization of specific viral polypeptide antigens such as coat or envelope antigens, or internal antigens, such as nucleic acid binding proteins, core antigens, and polynucleotide polymerase(s) may also be determined by, for example, determining whether the antigens are present as major or minor viral components, as well as by utilizing antibodies directed against the specific antigens encoded within isolated cDNAs as probes. This information is useful in the design of vaccines; for example, it may be

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preferable to include an exterior antigen in a vaccine preparation. Multivalent vaccines may be comprised of, for example, a polypeptide derived from the genome encoding a structural protein, for example, E, as well as a polypeptide from another portion of the genome, for example, a nonstructural or structural polypeptide.

#### 5 II.K. Cell Culture Systems and Animal Model Systems for HCV Replication

[0186] The suggestion that HCV is a flavivirus or flavi-like virus also provides information on methods for growing HCV. The term "flavi-like" means that the virus shows a significant amount of homology to the known conserved regions of flaviviruses and that the majority of the genome is a single ORF. Methods for culturing flaviviruses are known to those of skill in the art (See, for example, the reviews by Brinton (1986) and Stollar, V. (1980)). Generally, suitable cells or cell lines for culturing HCV may include those known to support Flavivirus replication, for example, the following: monkey kidney cell lines (e.g. MK<sub>2</sub>, VERO); porcine kidney cell lines (e.g. PS); baby hamster kidney cell lines (e.g. BHK); murine macrophage cell lines (e.g., P388D1, MK1, Mm1); human macrophage cell lines (e.g., U-937); human peripheral blood leukocytes; human adherent monocytes; hepatocytes or hepatocyte cell lines (e.g., HUH7, HEPG2); embryos or embryonic cells (e.g., chick embryo fibroblasts); or cell lines derived from invertebrates, preferably from insects (e.g. drosophila cell lines), or more preferably from arthropods, for example, mosquito cell lines (e.g., A. Albopictus, Aedes aegypti, Culex tritaeniorhynchus) or tick cell lines (e.g. RML-14 Dermacentor parumapertus).

[0187] It is possible that primary hepatocytes can be cultured, and then infected with HCV; or alternatively, the hepatocyte cultures could be derived from the livers of infected individuals (e.g., humans or chimpanzees). The latter case is an example of a cell which is infected *in vivo* being passaged *in vitro*. In addition, various immortalization methods can be used to obtain cell-lines derived from hepatocyte cultures. For example, primary liver cultures (before and after enrichment of the hepatocyte population) may be fused to a variety of cells to maintain stability. For example, also, cultures may be infected with transforming viruses, or transfected with transforming genes in order to create permanent or semipermanent cell lines. In addition, for example, cells in liver cultures may be fused to established cell lines (e.g., HepG2). Methods for cell fusion are known in the art, and include, for example, the use of fusion agents such as polyethylene glycol, Sendai Virus, and Epstein-Barr virus.

[0188] As discussed above, HCV is a Flavivirus or Flavi-like virus. Therefore, it is probable that HCV infection of cell lines may be accomplished by techniques known in the art for infecting cells with Flaviviruses. These include, for example, incubating the cells with viral preparations under conditions which allow viral entry into the cell. In addition, it may be possible to obtain viral production by transfecting the cells with isolated viral polynucleotides. It is known that Togavirus and Flavivirus, RNAs are infectious in a variety of vertebrate cell lines (Pfefferkorn and Shapiro (1974)); and in a mosquito cell line (Peleg (1969)). Methods for transfecting tissue culture cells with RNA duplexes, positive stranded RNAs, and DNAs (including cDNAs) are known in the art, and include, for example, techniques which use electroporation, and precipitation with DEAE-Dextran or calcium phosphate. An abundant source of HCV RNA can be obtained by performing *in vitro* transcription of an HCV cDNA corresponding to the complete genome. Transfection with this material, or with cloned HCV cDNA should result in viral replication and the *in vitro* propagation of the virus.

[0189] In addition to cultured cells, animal model systems may be used for viral replication; animal systems in which flaviviruses are known to those of skill in the art (See, for example, the review by Monath (1986)). Thus, HCV replication may occur not only in chimpanzees, but also in, for example, marmosets and suckling mice.

#### 40 II.L. Screening for Anti-Viral Agents for HCV

[0190] The availability of cell culture and animal model systems for HCV also makes possible screening for anti-viral agents which inhibit HCV replication, and particularly for those agents which preferentially allow cell growth and multiplication while inhibiting viral replication. These screening methods are known by those of skill in the art. Generally, the anti-viral agents are tested at a variety of concentrations, for their effect on preventing viral replication in cell culture systems which support viral replication, and then for an inhibition of infectivity or of viral pathogenicity (and a low level of toxicity) in an animal model system.

[0191] Methods and compositions for detecting HCV antigens and HCV polynucleotides are useful for screening of anti-viral agents in that they provide an alternative, and perhaps more sensitive means, for detecting the agent's effect on viral replication than the cell plaque assay or ID<sub>50</sub> assay. For example, the HCV-polynucleotide probes described herein may be used to quantitate the amount of viral nucleic acid produced in a cell culture. This could be accomplished, for example, by hybridization or competition hybridization of the infected cell nucleic acids with a labeled HCV-polynucleotide probe. For example, also, anti-HCV antibodies may be used to identify and quantitate HCV antigen(s) in the cell culture utilizing the immunoassays described herein. In addition, since it may be desirable to quantitate HCV antigens in the infected cell culture by a competition assay, the polypeptides encoded within the HCV cDNAs described herein are useful in these competition assays. Generally, a recombinant HCV polypeptide derived from the HCV cDNA would be labeled, and the inhibition of binding of this labeled polypeptide to an HCV polypeptide due to the antigen

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produced in the cell culture system would be monitored. Moreover, these techniques are particularly useful in cases where the HCV may be able to replicate in a cell line without causing cell death.

## II.M. Preparation of Attenuated Strains of HCV

[0192] In addition to the above, utilizing the tissue culture systems and/or animal model systems, it may be possible to isolate attenuated strains of HCV. These strains would be suitable for vaccines, or for the isolation of viral antigens. Attenuated strains are isolatable after multiple passages in cell culture and/or an animal model. Detection of an attenuated strain in an infected cell or individual is achievable by techniques known in the art, and could include, for example, the use of antibodies to one or more epitopes encoded in HCV as a probe or the use of a polynucleotide containing an HCV sequence of at least about 8 nucleotides as a probe. Alternatively, or in addition, an attenuated strain may be constructed utilizing the genomic information of HCV provided herein, and utilizing recombinant techniques. Generally, one would attempt to delete a region of the genome encoding, for example, a polypeptide related to pathogenicity, but which allows viral replication. In addition, the genome construction would allow the expression of an epitope which gives rise to neutralizing antibodies for HCV. The altered genome could then be utilized to transform cells which allow HCV replication, and the cells grown under conditions to allow viral replication. Attenuated HCV strains are useful not only for vaccine purposes, but also as sources for the commercial production of viral antigens, since the processing of these viruses would require less stringent protection measures for the employees involved in viral production and/or the production of viral products.

## III. General Methods

[0193] The general techniques used in extracting the genome from a virus, preparing and probing a cDNA library, sequencing clones, constructing expression vectors, transforming cells, performing immunological assays such as radioimmunoassays and ELISA assays, for growing cells in culture, and the like are known in the art and laboratory manuals are available describing these techniques. However, as a general guide, the following sets forth some sources currently available for such procedures, and for materials useful in carrying them out.

### III A. Hosts and Expression Control Sequences

[0194] Both prokaryotic and eukaryotic host cells may be used for expression of desired coding sequences when appropriate control sequences which are compatible with the designated host are used. Among prokaryotic hosts, *E. coli* is most frequently used. Expression control sequences for prokaryotes include promoters, optionally containing operator portions, and ribosome binding sites. Transfer vectors compatible with prokaryotic hosts are commonly derived from, for example, pBR322, a plasmid containing operons conferring ampicillin and tetracycline resistance, and the various pUC vectors, which also contain sequences conferring antibiotic resistance markers. These markers may be used to obtain successful transformants by selection. Commonly used prokaryotic control sequences include the Beta-lactamase (penicillinase) and lactose promoter systems (Chang et al. (1977)), the tryptophan (*trp*) promoter system (Goeddel et al. (1980)) and the lambda-derived  $P_L$  promoter and N gene ribosome binding site (Shimatake et al. (1981)) and the hybrid *tac* promoter (De Boer et al. (1983)) derived from sequences of the *trp* and *lac* UV5 promoters. The foregoing systems are particularly compatible with *E. coli*; if desired, other prokaryotic hosts such as strains of *Bacillus* or *Pseudomonas* may be used, with corresponding control sequences.

[0195] Eukaryotic hosts include yeast and mammalian cells in culture systems. *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* are the most commonly used yeast hosts, and are convenient fungal hosts. Yeast compatible vectors carry markers which permit selection of successful transformants by conferring prototrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Yeast compatible vectors may employ the 2 micron origin of replication (Broach et al. (1983)), the combination of CEN3 and ARS1 or other means for assuring replication, such as sequences which will result in incorporation of an appropriate fragment into the host cell genome. Control sequences for yeast vectors are known in the art and include promoters for the synthesis of glycolytic enzymes (Hess et al. (1968); Holland et al. (1978)), including the promoter for 3 phosphoglycerate kinase (Hitzeman (1980)). Terminators may also be included, such as those derived from the *enolase* gene (Holland (1981)). Particularly useful control systems are those which comprise the glyceraldehyde-3 phosphate dehydrogenase (GAPDH) promoter or alcohol dehydrogenase (ADH) regulatable promoter, terminators also derived from GAPDH, and if secretion is desired, leader sequence from yeast alpha factor. In addition, the transcriptional regulatory region and the transcriptional initiation region which are operably linked may be such that they are not naturally associated in the wild-type organism. These systems are described in detail in EPO 120,551, published October 3, 1984; EPO 116,201, published August 22, 1984; and EPO 164,556, published December 18, 1985, all of which are assigned to the herein assignee, and are hereby incorporated herein by reference.

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[0196] Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and a number of other cell lines. Suitable promoters for mammalian cells are also known in the art and include viral promoters such as that from Simian Virus 40 (SV40) (Fiers (1978)), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Mammalian cells may also require terminator sequences and poly A addition sequences; enhancer sequences which increase expression may also be included, and sequences which cause amplification of the gene may also be desirable. These sequences are known in the art. Vectors suitable for replication in mammalian cells may include viral replicons, or sequences which insure integration of the appropriate sequences encoding NANBV epitopes into the host genome.

III.B. Transformations

[0197] Transformation may be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus, and by direct uptake of the polynucleotide. The transformation procedure used depends upon the host to be transformed. For example, transformation of the *E. coli* host cells with lambda-gt11 containing BB-NANBV sequences is discussed in the Example section, infra. Bacterial transformation by direct uptake generally employs treatment with calcium or rubidium chloride (Cohen (1972); Maniatis (1982)). Yeast transformation by direct uptake may be carried out using the method of Hinnen et al. (1978). Mammalian transformations by direct uptake may be conducted using the calcium phosphate precipitation method of Graham and Van der Eb (1978), or the various known modifications thereof.

III.C. Vector Construction

[0198] Vector construction employs techniques which are known in the art. Site-specific DNA cleavage is performed by treating with suitable restriction enzymes under conditions which generally are specified by the manufacturer of these commercially available enzymes. In general, about 1 microgram of plasmid or DNA sequence is cleaved by 1 unit of enzyme in about 20 microliters buffer solution by incubation of 1-2 hr at 37° C. After incubation with the restriction enzyme, protein is removed by phenol/chloroform extraction and the DNA recovered by precipitation with ethanol. The cleaved fragments may be separated using polyacrylamide or agarose gel electrophoresis techniques, according to the general procedures found in *Methods in Enzymology* (1980) 65:499-560.

[0199] Sticky ended cleavage fragments may be blunt ended using *E. coli* DNA polymerase I (Klenow) in the presence of the appropriate deoxynucleotide triphosphates (dNTPs) present in the mixture. Treatment with S1 nuclease may also be used, resulting in the hydrolysis of any single stranded DNA portions.

[0200] Ligations are carried out using standard buffer and temperature conditions using T4 DNA ligase and ATP; sticky end ligations require less ATP and less ligase than blunt end ligations. When vector fragments are used as part of a ligation mixture, the vector fragment is often treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase to remove the 5'-phosphate and thus prevent religation of the vector; alternatively, restriction enzyme digestion of unwanted fragments can be used to prevent ligation.

[0201] Ligation mixtures are transformed into suitable cloning hosts, such as *E. coli*, and successful transformants selected by, for example, antibiotic resistance, and screened for the correct construction.

III.D. Construction of Desired DNA Sequences

[0202] Synthetic oligonucleotides may be prepared using an automated oligonucleotide synthesizer as described by Warner (1984). If desired the synthetic strands may be labeled with <sup>32</sup>P by treatment with polynucleotide kinase in the presence of <sup>32</sup>P-ATP, using standard conditions for the reaction.

[0203] DNA sequences, including those isolated from cDNA libraries, may be modified by known techniques, including, for example site directed mutagenesis, as described by Zoller (1982). Briefly, the DNA to be modified is packaged into phage as a single stranded sequence, and converted to a double stranded DNA with DNA polymerase using, as a primer, a synthetic oligonucleotide complementary to the portion of the DNA to be modified, and having the desired modification included in its own sequence. The resulting double stranded DNA is transformed into a phage supporting host bacterium. Cultures of the transformed bacteria, which contain replications of each strand of the phage, are plated in agar to obtain plaques. Theoretically, 50% of the new plaques contain phage having the mutated sequence, and the remaining 50% have the original sequence. Replicates of the plaques are hybridized to labeled synthetic probe at temperatures and conditions which permit hybridization with the correct strand, but not with the unmodified sequence. The sequences which have been identified by hybridization are recovered and cloned.

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III.E. Hybridization with Probe

[0204] DNA libraries may be probed using the procedure of Grunstein and Hogness (1975). Briefly, in this procedure, the DNA to be probed is immobilized on nitro-cellulose filters, denatured, and prehybridized with a buffer containing 0-50% formamide, 0.75 M NaCl, 75 mM Na citrate, 0.02% (wt/v) each of bovine serum albumin, polyvinyl pyrrolidone, and Ficoll, 50 mM Na Phosphate (pH 6.5), 0.1% SDS, and 100 micrograms/ml carrier denatured DNA. The percentage of formamide in the buffer, as well as the time and temperature conditions of the prehybridization and subsequent hybridization steps depends on the stringency required. Oligomeric probes which require lower stringency conditions are generally used with low percentages of formamide, lower temperatures, and longer hybridization times. Probes containing more than 30 or 40 nucleotides such as those derived from cDNA or genomic sequences generally employ higher temperatures, e.g., about 40-42°C, and a high percentage, e.g., 50%, formamide. Following prehybridization, 5'-32P-labeled oligonucleotide probe is added to the buffer, and the filters are incubated in this mixture under hybridization conditions. After washing, the treated filters are subjected to autoradiography to show the location of the hybridized probe; DNA in corresponding locations on the original agar plates is used as the source of the desired DNA.

III.F. Verification of Construction and Sequencing

[0205] For routine vector constructions, ligation mixtures are transformed into E. coli strain HB101 or other suitable host, and successful transformants selected by antibiotic resistance or other markers. Plasmids from the transformants are then prepared according to the method of Clewell et al. (1969), usually following chloramphenicol amplification (Clewell (1972)). The DNA is isolated and analyzed, usually by restriction enzyme analysis and/or sequencing. Sequencing may be by the dideoxy method of Sanger et al. (1977) as further described by Messing et al. (1981), or by the method of Maxam et al. (1980). Problems with band compression, which are sometimes observed in GC rich regions, were overcome by use of T-deazoguanosine according to Barr et al. (1986).

III.G. Enzyme Linked Immunosorbent Assay

[0206] The enzyme-linked immunosorbent assay (ELISA) can be used to measure either antigen or antibody concentrations. This method depends upon conjugation of an enzyme to either an antigen or an antibody, and uses the bound enzyme activity as a quantitative label. To measure antibody, the known antigen is fixed to a solid phase (e.g., a microplate or plastic cup), incubated with test serum dilutions, washed, incubated with anti-immunoglobulin labeled with an enzyme, and washed again. Enzymes suitable for labeling are known in the art, and include, for example, horseradish peroxidase. Enzyme activity bound to the solid phase is measured by adding the specific substrate, and determining product formation or substrate utilization colorimetrically. The enzyme activity bound is a direct function of the amount of antibody bound.

[0207] To measure antigen, a known specific antibody is fixed to the solid phase, the test material containing antigen is added, after an incubation the solid phase is washed, and a second enzyme-labeled antibody is added. After washing, substrate is added, and enzyme activity is estimated colorimetrically, and related to antigen concentration.

IV. Examples

[0208] Described below are examples of the present invention and related data, which are provided only for illustrative purposes, and not to limit the scope of the present invention. In light of the present disclosure, numerous embodiments within the scope of the claims will be apparent to those of ordinary skill in the art. The procedures set forth, for example, in Sections IV.A. may, if desired, be repeated but need not be, as techniques are available for construction of the desired nucleotide sequences based on the information provided by the invention. Expression is exemplified in E. coli; however, other systems are available as set forth more fully in Section III.A. Additional epitopes derived from the genomic structure may also be produced, and used to generate antibodies as set forth below.

IV.A. Preparation, Isolation and Sequencing of HCV cDNAIV.A.1. Preparation of HCV cDNA

[0209] The source of NANB agent was a plasma pool derived from a chimpanzee with chronic NANBH. The chimpanzee had been experimentally infected with blood from another chimpanzee with chronic NANBH resulting from infection with HCV in a contaminated batch of factor 8 concentrate derived from pooled human sera. The chimpanzee plasma pool was made by combining many individual plasma samples containing high levels of alanine aminotransferase activity; this activity results from hepatic injury due to the HCV infection. Since 1 ml of a 10<sup>-6</sup> dilution of this

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pooled serum given i.v. caused NANBH in another chimpanzee, its CID was at least  $10^6$ /ml, i.e., it had a high infectious virus titer.

[0210] A cDNA library from the high titer plasma pool was generated as follows. First, viral particles were isolated from the plasma; a 90 ml aliquot was diluted with 310 ml of a solution containing 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 100 mM NaCl. Debris was removed by centrifugation for 20 min at  $15,000 \times g$  at  $20^\circ\text{C}$ . Viral particles in the resulting supernatant were then pelleted by centrifugation in a Beckman SW28 rotor at 28,000 rpm for 5 hours at  $20^\circ\text{C}$ . To release the viral genome, the particles were disrupted by suspending the pellets in 15 ml solution containing 1% sodium dodecyl sulfate (SDS), 10 mM EDTA, 10 mM Tris-HCl, pH 7.5, also containing 2 mg/ml proteinase K, followed by incubation at  $45^\circ\text{C}$  for 90 min. Nucleic acids were isolated by adding 0.8 micrograms MS2 bacteriophage RNA as carrier, and extracting the mixture four times with a 1:1 mixture of phenol:chloroform (phenol saturated with 0.5M Tris-HCl, pH 7.5, 0.1% (v/v) betamercaptoethanol, 0.1% (w/v) hydroxyquinolone, followed by extraction two times with chloroform. The aqueous phase was concentrated with 1-butanol prior to precipitation with 2.5 volumes absolute ethanol overnight at  $-20^\circ\text{C}$ . Nucleic acid was recovered by centrifugation in a Beckman SW41 rotor at 40,000 rpm for 90 min at  $4^\circ\text{C}$ , and dissolved in water that had been treated with 0.05% (v/v) diethylpyrocarbonate and autoclaved.

[0211] Nucleic acid obtained by the above procedure ( $<2$  micrograms) was denatured with 17.5 mM  $\text{CH}_3\text{HgOH}$ ; cDNA was synthesized using this denatured nucleic acid as template, and was cloned into the EcoRI site of phage lambda-gt11 using methods described by Huynh (1985), except that random primers replaced oligo(dT) 12-18 during the synthesis of the first cDNA strand by reverse transcriptase (Taylor et al. (1976)). The resulting double stranded cDNAs were fractionated according to size on a Sepharose CL-4B column; eluted material of approximate mean size 400, 300, 200, and 100 base-pairs were pooled into cDNA pools 1, 2, 3, and 4, respectively. The lambda-gt11 cDNA library was generated from the cDNA in pool 3.

[0212] The lambda-gt11 cDNA library generated from pool 3 was screened for epitopes that could bind specifically with serum derived from a patient who had previously experienced NANBH. About  $10^6$  phage were screened with patient sera using the methods of Huynh et al. (1985), except that bound human antibody was detected with sheep anti-human Ig antisera that had been radio-labeled with  $^{125}\text{I}$ . Five positive phages were identified and purified. The five positive phages were then tested for specificity of binding to sera from 8 different humans previously infected with the NANBH agent, using the same method. Four of the phage encoded a polypeptide that reacted immunologically with only one human serum, i.e., the one that was used for primary screening of the phage library. The fifth phage (5-1-1) encoded a polypeptide that reacted immunologically with 5 of 8 of the sera tested. Moreover, this polypeptide did not react immunologically with sera from 7 normal blood donors. Therefore, it appears that clone 5-1-1 encodes a polypeptide which is specifically recognized immunologically by sera from NANB patients.

#### IV.A.2. Sequences of the HCV cDNA in Recombinant Phage 5-1-1, and of the Polypeptide Encoded Within the Sequence.

[0213] The cDNA in recombinant phage 5-1-1 was sequenced by the method of Sanger et al. (1977). Essentially, the cDNA was excised with EcoRI, isolated by size fractionation using gel electrophoresis. The EcoRI restriction fragments were subcloned into the M13 vectors, mp18 and mp19 (Messing (1983)) and sequenced using the dideoxycytosine termination method of Sanger et al. (1977). The sequence obtained is shown in Fig. 1.

[0214] The polypeptide encoded in Fig. 1 that is encoded in the HCV cDNA is in the same translational frame as the N-terminal beta-galactosidase moiety to which it is fused. As shown in Section IV.A., the translational open reading frame (ORF) of 5-1-1 encodes epitope(s) specifically recognized by sera from patients and chimpanzees with NANBH infections.

#### IV.A.3. Isolation of Overlapping HCV cDNA to cDNA in Clone 5-1-1.

[0215] Overlapping HCV cDNA to the cDNA in clone 5-1-1 was obtained by screening the same lambda-gt11 library, created as described in Section IV.A.1., with a synthetic polynucleotide derived from the sequence of the HCV cDNA in clones 5-1-1, as shown in Fig. 1. The sequence of the polynucleotide used for screening was:

5'-TCC CTT GCT CGA TGT ACG GTA AGT GCT GAG AGC  
ACT CTT CCA TCT CAT CGA ACT CTC GGT AGA GGA CTT CCC TGT  
CAG GT-3'.

The lambda-gt11 library was screened with this probe, using the method described in Huynh (1985). Approximately 1 in 50,000 clones hybridized with the probe. Three clones which contained cDNAs which hybridized with the synthetic

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probe have been numbered 81, 1-2, and 91.

#### IV.A.4. Nucleotide Sequences of Overlapping HCV cDNAs to cDNA in Clone 5-1-1.

5 [0216] The nucleotide sequences of the three cDNAs in clones 81, 1-2, and 91 were determined essentially as in Section IV.A.2. The sequences of these clones relative to the HCV cDNA sequence in phage 5-1-1 is shown in Fig. 2, which shows the strand encoding the detected HCV epitope, and where the homologies in the nucleotide sequences are indicated by vertical lines between the sequences.

10 [0217] The sequences of the cloned HCV cDNAs are highly homologous in the overlapping regions (see Fig. 2). However, there are differences in two regions. Nucleotide 67 in clone 1-2 is a thymidine, whereas the other three clones contain a cytidine residue in this position. It should be noted, however, that the same amino acid is encoded when either C or T occupies this position.

15 [0218] The second difference is that clone 5-1-1 contains 28 base pairs which are not present in the other three clones. These base pairs occur at the start of the cDNA sequence in 5-1-1, and are indicated by small letters. Based on radioimmunoassay data, which is discussed infra in Section IV.D., it is possible that an HCV epitope may be encoded in this 28 bp region.

[0219] The absence of the 28 base pairs of 5-1-1 from clones 81, 1-2, and 91 may mean that the cDNA in these clones were derived from defective HCV genomes; alternatively, the 28 bp region could be a terminal artifact in clone 5-1-1.

20 [0220] The sequences of small letters in the nucleotide sequence of clones 81 and 91 simply indicate that these sequences have not been found in other cDNAs because cDNAs overlapping these regions were not yet isolated.

[0221] A composite HCV cDNA sequence derived from overlapping cDNAs in clones 5-1-1, 81, 1-2 and 91 is shown in Fig. 3. However, in this figure the unique 28 base pairs of clone 5-1-1 are omitted. The figure also shows the sequence of the polypeptide encoded within the ORF of the composite HCV cDNA.

#### IV.A.5. Isolation of Overlapping HCV cDNAs to cDNA in Clone 81.

30 [0222] The isolation of HCV cDNA sequences upstream of, and which overlap those in clone 81 cDNA was accomplished as follows. The lambda-gt11 cDNA library prepared as described in Section IV.A.1. was screened by hybridization with a synthetic polynucleotide probe which was homologous to a 5' terminal sequence of clone 81. The sequence of clone 81 is presented in Fig. 4. The sequence of the synthetic polynucleotide used for screening was:

5, CTG TCA GGT ATG ATT GCC GGC TTC CCG GAC 3'.

35 The methods were essentially as described in Huynh (1985), except that the library filters were given two washes under stringent conditions, i.e., the washes were in 5 x SSC, 0.1% SDS at 55°C for 30 minutes each. Approximately 1 in 50,000 clones hybridized with the probe. A positive recombinant phage which contained cDNA which hybridized with the sequence was isolated and purified. This phage has been numbered clone 36.

40 [0223] Downstream cDNA sequences, which overlaps the carboxyl-end sequences in clone 81 cDNA were isolated using a procedure similar to that for the isolation of upstream cDNA sequences, except that a synthetic oligonucleotide probe was prepared which is homologous to a 3' terminal sequence of clone 81. The sequence of the synthetic polynucleotide used for screening was:

45 6' TTT GGC TAG TGG TTA GTG GGC TGG TGA CAG 3'

A positive recombinant phage, which contained cDNA which hybridized with this latter sequence was isolated and purified, and has been numbered clone 32.

#### IV.A.6. Nucleotide Sequence of HCV cDNA in Clone 36.

50 [0224] The nucleotide sequence of the cDNA in clone 36 was determined essentially as described in Section IV.A.2. The double-stranded sequence of this cDNA, its region of overlap with the HCV cDNA in clone 81, and the polypeptide encoded by the ORF are shown in Fig. 5.

55 [0225] The ORF in clone 36 is in the same translational frame as the HCV antigen encoded in clone 81. Thus, in combination, the ORFs in clones 36 and 81 encode a polypeptide that represents part of a large HCV antigen. The sequence of this putative HCV polypeptide and the double stranded DNA sequence encoding it, which is derived from the combined ORFs of the HCV cDNAs of clones 36 and 81, is shown in Fig. 6.

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IV.A.7 Nucleotide Sequences of HCV cDNA In Clone 32

[0226] The nucleotide sequence of the cDNA in clone 32 was determined essentially as was that described in Section IV.A.2 for the sequence of clone 5-1-1. The sequence data indicated that the cDNA in clone 32 recombinant phage was derived from two different sources. One fragment of the cDNA was comprised of 418 nucleotides derived from the HCV genome; the other fragment was comprised of 172 nucleotides derived from the bacteriophage MS2 genome, which had been used as a carrier during the preparation of the lambda gt11 plasma cDNA library.

[0227] The sequence of the cDNA in clone 32 corresponding to that of the HCV genome is shown in Fig. 7. The region of the sequences that overlaps that of clone 81, and the polypeptide encoded by the ORF are also indicated in the figure. This sequence contains one continuous ORF that is in the same translational frame as the HCV antigen encoded by clone 81.

IV.A.8 Isolation of Overlapping HCV cDNA to cDNA In Clone 36

[0228] The isolation of HCV cDNA sequences upstream of, and which overlap those in clone 36 cDNA was accomplished as described in Section IV.A.5, for those which overlap clone 81 cDNA, except that the synthetic polynucleotide was based on the 5'-region of clone 36. The sequence of the synthetic polynucleotide used for screening was:

5' AAG CCA CCG TGT GCG CTA GGG CTC AAG CCC 3'

Approximately 1 in 50,000 clones hybridized with the probe. The isolated, purified clone of recombinant phage which contained cDNA which hybridized to this sequence was named clone 35.

IV.A.9 Nucleotide Sequence of HCV cDNA In Clone 35

[0229] The nucleotide sequence of the cDNA in clone 35 was determined essentially as described in Section IV.A.2. The sequence, its region of overlap with that of the cDNA in clone 36, and the putative polypeptide encoded therein, are shown in Fig. 8.

[0230] Clone 35 apparently contains a single, continuous ORF that encodes a polypeptide in the same translational frame as that encoded by clone 36, clone 81, and clone 32. Fig. 9 shows the sequence of the long continuous ORF that extends through clones 35, 36, 81, and 32, along with the putative HCV polypeptide encoded therein. This combined sequence has been confirmed using other independent cDNA clones derived from the same lambda gt11 cDNA library.

IV-A-10. Isolation of Overlapping HCV cDNA to cDNA in Clone 35

[0231] The isolation of HCV cDNA sequences upstream of, and which overlap those in clone 35 cDNA was accomplished as described in Section IV.A.8, for those which overlap clone 36 cDNA, except that the synthetic polynucleotide was based on the 5'-region of clone 35. The sequence of the synthetic polynucleotide used for screening was:

5' CAG GAT GCT GTC TCC CGC ACT CAA CGT 3'

Approximately 1 in 50,000 clones hybridized with the probe. The isolated, purified clone of recombinant phage which contained cDNA which hybridized to this sequence was named clone 37b.

IV.A.11. Nucleotide Sequence of HCV In Clone 37b

[0232] The nucleotide sequence of the cDNA in clone 37b was determined essentially as described in Section IV.A.2. The sequence, its region of overlap with that of the cDNA in clone 35, and the putative polypeptide encoded therein, are shown in Fig. 10.

[0233] The 5'-terminal nucleotide of clone 35 is a T, whereas the corresponding nucleotide in clone 37b is an A. The cDNAs from three other independent clones which were isolated during the procedure in which clone 37b was isolated, described in Section IV.A.10, have also been sequenced. The cDNAs from these clones also contain an A in this position. Thus, the 5'-terminal T in clone 35 may be an artefact of the cloning procedure. It is known that artefacts often arise at the 5'-termini of cDNA molecules.

[0234] Clone 37b apparently contains one continuous ORF which encodes a polypeptide which is a continuation of the polypeptide encoded in the ORF which extends through the overlapping clones 35, 36, 81 and 32.

**EP 0 318 216 B2****IV.A.12 Isolation of Overlapping HCV cDNA to cDNA in Clone 32**

[0235] The isolation of HCV cDNA sequences downstream of clone 32 was accomplished as follows. First, clone cla was isolated utilizing a synthetic hybridization probe which was based on the nucleotide sequence of the HCV cDNA sequence in clone 32. The method was essentially that described in Section IV.A.5, except that the sequence of the synthetic probe was:

5' AGT GCA GTG GAT GAA CCG GCT GAT AGC CTT 3'.

Utilizing the nucleotide sequence from clone cla, another synthetic nucleotide was synthesized which had the sequence:

5' TCC TGA GGC GAC TGC ACC AGT GGA TAA GCT 3'.

Screening of the lambda gt11 library using the clone cla derived sequence as probe yielded approximately 1 in 50,000 positive colonies. An isolated, purified clone which hybridized with this probe was named clone 33b.

**IV.A.13 Nucleotide Sequence of HCV cDNA in Clone 33b**

[0236] The nucleotide sequence of the cDNA in clone 33b was determined essentially as described in Section IV.A.2. The sequence, its region of overlap with that of the cDNA in clone 32, and the putative polypeptide encoded therein, are shown in Fig. 11.

[0237] Clone 33b apparently contains one continuous ORF which is an extension of the ORFs in overlapping clones 37b, 35, 36, 81 and 32. The polypeptide encoded in clone 33b is in the same translational frame as that encoded in the extended ORF of these overlapping clones.

**IV.A.14 Isolation of Overlapping HCV cDNAs to cDNA Clone 37b and to cDNA in Clone 33b**

[0238] In order to isolate HCV cDNAs which overlap the cDNAs in clone 37b and in clone 33b, the following synthetic oligonucleotide probes, which were derived from the cDNAs in those clones, were used to screen the lambda gt11 library, using essentially the method described in Section IV.A.3. The probes used were:

5' CAG GAT GCT GTC TCC CGC ACT CAA CGT C 3'

and

5' TCC TGA GGC GAC TGC ACC AGT GGA TAA GCT 3'

to detect colonies containing HCV cDNA sequences which overlap those in clones 37b and 33b, respectively. Approximately 1 in 50,000 colonies were detected with each probe. A clone which contained cDNA which was upstream of, and which overlapped the cDNA in clone 37b, was named clone 40b. A clone which contained cDNA which was downstream of, and which overlapped the cDNA in clone 33b was named clone 25c.

**IV.A.15 Nucleotide Sequences of HCV cDNA in clone 40b and in clone 25c**

[0239] The nucleotide sequences of the cDNAs in clone 40b and in clone 25c were determined essentially as described in Section IV.A.2. The sequences of 40b and 25c, their regions of overlap with the cDNAs in clones 37b and 33b, and the putative polypeptides encoded therein, are shown in Fig. 12 (clone 40b) and Fig. 13 (clone 25c).

[0240] The 5'-terminal nucleotide of clone 40b is a G. However, the cDNAs from five other independent clones which were isolated during the procedure in which clone 40b was isolated, described in Section IV.A.14, have also been sequenced. The cDNAs from these clones also contain a T in this position. Thus, the G may represent a cloning artifact (see the discussion in Section IV.A.11).

[0241] The 5'-terminus of clone 25c is ACT, but the sequence of this region in clone cla (sequence not shown), and

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In clone 33b is TCA. This difference may also represent a cloning artifact, as may the 28 extra 5'-terminal nucleotides in clone 5-1-1.

[0242] Clones 40b and 25c each apparently contain an ORF which is an extension of the continuous ORF in the previously sequenced clones. The nucleotide sequence of the ORF extending through clones 40b, 37b, 35, 36, 81, 32, 33b, and 25c, and the amino acid sequence of the putative polypeptide encoded therein, are shown in Fig. 14. In the figure, the potential artifacts have been omitted from the sequence, and instead, the corresponding sequences in non-5'-terminal regions of multiple overlapping clones are shown.

#### IV.A.16. Preparation of a Composite HCV cDNA from the cDNAs in Clones 36, 81, and 32

[0243] The composite HCV cDNA, C100, was constructed as follows. First the cDNAs from the clones 36, 81, and 32 were excised with EcoRI. The EcoRI fragment of cDNA from each clone was cloned individually into the EcoRI site of the vector pGEM3-blue (Promega Biotec). The resulting recombinant vectors which contained the cDNAs from clones 36, 81, and 32 were named pGEM3-blue/36, pGEM3-blue/81, and pGEM3-blue/32, respectively. The appropriately oriented recombinant of pGEM3-blue/81 was digested with NaeI and NarI, and the large (~2850bp) fragment was purified and ligated with the small (~570bp) NaeI/NarI purified restriction fragment from pGEM3-blue/36. This composite of the cDNAs from clones 36 and 81 was used to generate another pGEM3-blue vector containing the continuous HCV ORF contained within the overlapping cDNA within these clones. This new plasmid was then digested with PvuII and EcoRI to release a fragment of approximately 680bp, which was then ligated with the small (580bp) PvuII/EcoRI fragment isolated from the appropriately oriented pGEM3-blue/32 plasmid, and the composite cDNA from clones 36, 81, and 32 was ligated into the EcoRI linearized vector pSODc1, which is described in Section IV.B.1, and which was used to express clone 5-1-1 in bacteria. Recombinants containing the ~1270bp EcoRI fragment of composite HCV cDNA (C100) were selected, and the cDNA from the plasmids was excised with EcoRI and purified.

#### IV.A.17. Isolation and Nucleotide Sequences of HCV cDNAs in Clones 14i, 11b, 7f, 7e, 8h, 33c, 14c, 8f, 33f, 33g, and 39c

[0244] The HCV cDNAs in clones 14i, 11b, 7f, 7e, 8h, 33c, 14c, 8f, 33f, 33g, and 39c were isolated by the technique of isolating overlapping cDNA fragments from the lambda gt11 library of HCV cDNAs described in Section IV.A.1. The technique used was essentially as described in Section IV.A.3., except that the probes used were designed from the nucleotide sequence of the last isolated clones from the 5' and the 3' end of the combined HCV sequences. The frequency of clones which hybridized with the probes described below was approximately 1 in 50,000 in each case.

[0245] The nucleotide sequences of the HCV cDNAs in clones 14i, 7f, 7e, 8h, 33c, 14c, 8f, 33f, 33g, and 39c were determined essentially as described in Section IV.A.2., except that the cDNA excised from these phages were substituted for the cDNA isolated from clone 5-1-1.

[0246] Clone 33c was isolated using a hybridization probe based on the sequence of nucleotides in clone 40b. The nucleotide sequence of clone 40b is presented in Fig. 12. The nucleotide sequence of the probe used to isolate 33c was:

5' ATC AGG ACC GGG GTG AGA ACA ATT ACC ACT 3'

The sequence of the HCV cDNA in clone 33c, and the overlap with that in clone 40b, is shown in Fig. 15, which also shows the amino acids encoded therein.

[0247] Clone 8h was isolated using a probe based on the sequence of nucleotides in clone 33c. The nucleotide sequence of the probe was

5' AGA GAC AAC CAT GAG GTC CCC GGT GTT C 3'.

The sequence of the HCV cDNA in clone 8h, and the overlap with that in clone 33c, and the amino acids encoded therein, are shown in Fig. 16.

[0248] Clone 7e was isolated using a probe based on the sequence of nucleotides in clone 8h. The nucleotide sequence of the probe was

5' TCG GAC CTT TAC CTG GTC ACG AGG CAC 3'.

The sequence of HCV cDNA in clone 7e, the overlap with clone 8h, and the amino acids encoded therein, are shown in Fig. 17.

[0249] Clone 14c was isolated with a probe based on the sequence of nucleotides in clone 25c. The sequence of clone 25c is shown in Fig. 13. The probe in the isolation of clone 14c had the sequence

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5' ACC TTC CCC ATT AAT GCC TAC ACC ACG GGC 3'.

The sequence of HCV cDNA in clone 14c, its overlap with that in clone 25c, and the amino acids encoded therein are shown in Fig. 18.

5 [0250] Clone 8f was isolated using a probe based on the sequence of nucleotides in clone 14c. The nucleotide sequence of the probe was

5' TCC ATC TCT CAA GGC AAC TTG CAC CGC TAA 3'.

10 The sequence of HCV cDNA in clone 8f, its overlap with that in clone 14c, and the amino acids encoded therein are shown in Fig. 19.

[0251] Clone 33f was isolated using a probe based on the nucleotide sequence present in clone 8f. The nucleotide sequence of the probe was

15 5' TCC ATG GCT GTC CGC TTC CAC CTC CAA AGT 3'.

The sequence of HCV cDNA in clone 33f, its overlap with that in clone 8f, and the amino acids encoded therein are shown in Fig. 20.

20 [0252] Clone 33g was isolated using a probe based on the sequence of nucleotides in clone 33f. The nucleotide sequence of the probe was

5' GCG ACA ATA CGA CAA CAT CCT CTG AGC CCG 3'.

25 The sequence of HCV cDNA in clone 33g, its overlap with that in clone 33f, and the amino acids encoded therein are shown in Fig. 21.

[0253] Clone 7f was isolated using a probe based on the sequence of nucleotides in clone 7e. The nucleotide sequence of the probe was

30 5' AGC AGA CAA GGG GCC TCC TAG GGT GCA TAA T 3'.

The sequence of HCV cDNA in clone 7f, its overlap with clone 7e, and the amino acids encoded therein are shown in Fig. 22.

[0254] Clone 11b was isolated using a probe based on the sequence of clone 7f. The nucleotide sequence of the probe was

35 5' CAC CTA TGT TTA TAA CCA TCT CAC TCC TCT 3'.

The sequence of HCV cDNA in clone 11b, its overlap with clone 7f, and the amino acids encoded therein are shown in Fig. 23.

40 [0255] Clone 14i was isolated using a probe based on the sequence of nucleotides in clone 11b. The nucleotide sequence of the probe was

5' CTC TGT CAC CAT ATT ACA AGC GCT ATA TCA 3'.

45 The sequence of HCV cDNA in clone 14i, its overlap with 11b, and the amino acids encoded therein are shown in Fig. 24.

[0256] Clone 39c was isolated using a probe based on the sequence of nucleotides in clone 33g. The nucleotide sequence of the probe was

50 5' CTC GTT GCT ACG TCA CCA CAA TTT GGT GTA 3'.

The sequence of HCV cDNA in clone 39c, its overlap with clone 33g, and the amino acids encoded therein are shown in Fig. 25.

#### IV.A.18. The Composite HCV cDNA Sequence Derived from Isolated Clones Containing HCV cDNA

55 [0257] The HCV cDNA sequences in the isolated clones described supra have been aligned to create a composite HCV cDNA sequence. The isolated clones, aligned in the 5' to 3' direction are: 14i, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, and 39c.

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[0258] A composite HCV cDNA sequence derived from the isolated clones, and the amino acids encoded therein, is shown in Fig. 26.

[0259] In creating the composite sequence the following sequence heterogeneities have been considered. Clone 33c contains an HCV cDNA of 800 base pairs, which overlaps the cDNAs in clones 40b and 37c. In clone 33c, as well as in 5 other overlapping clones, nucleotide #789 is a G. However, in clone 37b (see Section IV.A.11), the corresponding nucleotide is an A. This sequence difference creates an apparent heterogeneity in the amino acids encoded therein, which would be either CYS or TYR, for G or A, respectively. This heterogeneity may have important ramifications in terms of protein folding.

[0260] Nucleotide residue #2 in clone 8h HCV cDNA is a T. However, as shown infra, the corresponding residue in clone 7e is an A; moreover, an A in this position is also found in 3 other isolated overlapping clones. Thus, the T residue in clone 8h may represent a cloning artifact. Therefore, in Fig. 26, the residue in this position is designated as an A.

[0261] The 3'-terminal nucleotide in clone 8f HCV cDNA is a G. However, the corresponding residue in clone 33f, and in 2 other overlapping clones is a T. Therefore, in Fig. 26, the residue in this position is designated as a T.

[0262] The 3'-terminal sequence in clone 33f HCV cDNA is TTGC. However, the corresponding sequence in clone 33g and in 2 other overlapping clones is ATTC. Therefore, in Fig. 26, the corresponding region is represented as ATTC.

[0263] Nucleotide residue #4 in clone 33g HCV cDNA is a T. However, in clone 33f and in 2 other overlapping clones the corresponding residue is an A. Therefore, in Fig. 26, the corresponding residue is designated as an A.

[0264] The 3'-terminus of clone 14i is an AA, whereas the corresponding dinucleotide in clone 11b, and in three other clones, is TA. Therefore, in Fig. 26, the TA residue is depicted.

[0265] The resolution of other sequence heterogeneities is discussed supra.

[0266] An examination of the composite HCV cDNA indicates that it contains one large ORF. This suggests that the viral genome is translated into a large polypeptide which is processed concomitant with, or subsequent to translation.

#### IV.A.19. Isolation and Nucleotide Sequences of HCV cDNAs in Clones 12f, 35f, 19g, 26g, and 15e

[0267] The HCV cDNAs in clones 12f, 35f, 19g, 26g, and 15e were isolated essentially by the technique described in Section IV.A.17, except that the probes were as indicated below. The frequency of clones which hybridized with the probes was approximately 1 in 50,000 in each case. The nucleotide sequences of the HCV cDNAs in these clones were determined essentially as described in Section IV.A.2., except that the cDNA from the indicated clones were substituted for the cDNA isolated from clone 5-1-1.

[0268] The isolation of clone 12f, which contains cDNA upstream of the HCV cDNA in Fig. 26, was accomplished using a hybridization probe based on the sequence of nucleotides in clone 14i. The nucleotide sequence of the probe was

5' TGC TTG TGG ATG ATG CTA CTC ATA TCC CAA 3'.

The HCV cDNA sequence of clone 12f, its overlap with clone 14i, and the amino acids encoded therein are shown in Fig. 27.

[0269] The isolation of clone 35f, which contains cDNA downstream of the HCV cDNA in Fig. 26, was accomplished using a hybridization probe based on the sequence of nucleotides in clone 39c. The nucleotide sequence of the probe was

5' AGC AGC GGC GTC AAA AGT GAA GGC TAA CTT 3'.

The sequence of clone 35f, its overlap with the sequence in clone 39c, and the amino acids encoded therein are shown in Fig. 28.

[0270] The isolation of clone 19g was accomplished using a hybridization probe based on the 3' sequence of clone 35f. The nucleotide sequence of the probe was

5' TTC TCG TAT GAT ACC CGC TGC TTT GAC TCC 3'.

The HCV cDNA sequence of clone 19g, its overlap with the sequence in clone 35f, and the amino acids encoded therein are shown in Fig. 29.

[0271] The isolation of clone 26g was accomplished using a hybridization probe based on the 3' sequence of clone 19g. The nucleotide sequence of the probe was

5' TGT GTG GCG ACG ACT TAG TCG TTA TCT GTG 3'.

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The HCV cDNA sequence of clone 26g, its overlap with the sequence in clone 19g, and the amino acids encoded therein are shown in Fig. 30.

[0272] Clone 15e was isolated using a hybridization probe based on the 3' sequence of clone 26g. The nucleotide sequence of the probe was

5' CAC ACT CCA GTC AAT TCC TGG CTA GGC AAC 3'.

The HCV cDNA sequence of clone 15e, its overlap with the sequence in clone 26g, and the amino acids encoded therein are shown in Fig. 31.

[0273] The clones described in this Section have been deposited with the ATCC under the terms and conditions described in Section II A., and have been assigned the following Accession Numbers.

lambda-gt11	ATCC No.	Deposit Date
clone 12f	40514	10 November 1988
clone 35f	40511	10 November 1988
clone 15e	40513	10 November 1988
clone k9-1	40512	10 November 1988

[0274] The HCV cDNA sequences in the isolated clones described supra. have been aligned to create a composite HCV cDNA sequence. The isolated clones, aligned in the 5, to 3' direction are: 12f, 14i, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f 33f, 33g, 39c, 35f, 19g, 26g, and 15e.

[0275] A composite HCV cDNA sequence derived from the isolated clones, and the amino acids encoded therein, is shown in Fig. 32.

#### IV.A.20. Alternative Method of Isolating cDNA Sequences Upstream of the HCV cDNA Sequence in Clone 12f

[0276] Based on the most 5' HCV sequence in Fig. 32, which is derived from the HCV cDNA in clone 12f, small synthetic oligonucleotide primers of reverse transcriptase are synthesized and used to bind to the corresponding sequence in HCV genomic RNA, to prime reverse transcription of the upstream sequences. The primer sequences are proximal to the known 5'-terminal sequence of clone 12f, but sufficiently downstream to allow the design of probe sequences upstream of the primer sequences. Known standard methods of priming and cloning are used. The resulting cDNA libraries are screened with sequences upstream of the priming sites (as deduced from the elucidated sequence in clone 12f). The HCV genomic RNA is obtained from either plasma or liver samples from chimpanzees with NANBH, or from analogous samples from humans with NANBH.

#### IV.A.21. Alternative Method Utilizing Tailing to Isolate Sequences from the 5'-Terminal Region of the HCV Genome

[0277] In order to isolate the extreme 5'-terminal sequences of the HCV RNA genome, the cDNA product of the first round of reverse transcription, which is duplexed with the template RNA, is tailed with oligo C. This is accomplished by incubating the product with terminal transferase in the presence of CTP. The second round of cDNA synthesis, which yields the complement of the first strand of cDNA, is accomplished utilizing oligo G as a primer for the reverse transcriptase reaction. The sources of genomic HCV RNA are as described in Section IV.A.20. The methods for tailing with terminal transferase, and for the reverse transcriptase reactions are as in Maniatis et al. (1982). The cDNA products are then cloned, screened, and sequenced.

#### IV.A.22. Alternative Method Utilizing Tailing to Isolate Sequences from the 3'-Terminal Region of the HCV Genome

[0278] This method is based on previously used methods for cloning cDNAs of Flavivirus RNA. In this method, the RNA is subjected to denaturing conditions to remove secondary structures at the 3'-terminus, and is then tailed with Poly A polymerase using rATP as a substrate. Reverse transcription of the poly A tailed RNA is catalyzed by reverse transcriptase, utilizing oligo dT as a primer. The second strands of cDNA are synthesized, the cDNA products are cloned, screened, and sequenced.

#### IV.A.23 Creation of Lambda-gt11 HCV cDNA Libraries Containing Larger cDNA Inserts

[0279] The method used to create and screen the Lambda gt11 libraries are essentially as described in Section IV.

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A.1., except that the library is generated from a pool of larger size cDNAs eluted from the Sepharose CL-4B column.

IV.A.24. Creation of HCV cDNA Libraries Using Synthetic Oligomers as Primers

[0280] New HCV cDNA libraries have been prepared from the RNA derived from the infectious chimpanzee plasma pool described in Section IV.A.1., and from the poly A<sup>+</sup> RNA fraction derived from the liver of this infected animal. The cDNA was constructed essentially as described by Gubler and Hoffman (1983), except that the primers for the first cDNA strand synthesis were two synthetic oligomers based on the sequence of the HCV genome described supra. Primers based on the sequence of clone 11 b and 7e were, respectively,

5' CTG GCT TGA AGA ATC 3'

and

5' AGT TAG GCT GGT GAT TAT GC 3'.

The resulting cDNAs were cloned into lambda bacteriophage vectors, and screened with various other synthetic oligomers, whose sequences were based on the HCV sequence in Fig. 32.

IV.B. Expression of Polypeptides Encoded Within HCV cDNAs and Identification of the Expressed Products as HCV Induced Antigens.IV.B.1. Expression of the Polypeptide Encoded in Clone 5-1-1.

[0281] The HCV polypeptide encoded within clone 5-1-1 (see Section IV.A.2., supra) was expressed as a fusion polypeptide with superoxide dismutase (SOD). This was accomplished by subcloning the clone 5-1-1 cDNA insert into the expression vector pSODcf1 (Stelmer et al. (1986)) as follows.

[0282] First, DNA isolated from pSODcf1 was treated with BamHI and EcoRI, and the following linker was ligated into the linear DNA created by the restriction enzymes:

5' GAT CCT GGA ATT CTG ATA A 3'  
3' GA CCT TAA GAC TAT TTT AA 5'

After cloning, the plasmid containing the insert was isolated.

[0283] Plasmid containing the insert was restricted with EcoRI. The HCV cDNA insert in clone 5-1-1 was excised with EcoRI, and ligated into this EcoRI linearized plasmid DNA. The DNA mixture was used to transform *E. coli* strain D1210 (Sadler et al. (1980)). Recombinants with the 5-1-1 cDNA in the correct orientation for expression of the ORF shown in Fig. 1 were identified by restriction mapping and nucleotide sequencing.

[0284] Recombinant bacteria from one clone were induced to express the SOD-NANB<sub>5-1-1</sub> polypeptide by growing the bacteria in the presence of IPTG.

IV.B.2. Expression of the Polypeptide Encoded in Clone 81.

[0285] The HCV cDNA contained within clone 81 was expressed as a SOD-NANB<sub>81</sub> fusion polypeptide. The method for preparing the vector encoding this fusion polypeptide was analogous to that used for the creation of the vector encoding SOD-NANB<sub>5-1-1</sub>, except that the source of the HCV cDNA was clone 81, which was isolated as described in Section IV.A.3, and for which the cDNA sequence was determined as described in Section IV.A.4. The nucleotide sequence of the HCV cDNA in clone 81, and the putative amino acid sequence of the polypeptide encoded therein are shown in Fig. 4.

[0286] The HCV cDNA insert in clone 81 was excised with EcoRI, and ligated into the pSODcf1 which contained the linker (see IV.B.1.) and which was linearized by treatment with EcoRI. The DNA mixture was used to transform *E. coli*

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strain D1210. Recombinants with the clone 81 HCV cDNA in the correct orientation for expression of the ORF shown in Fig. 4 were identified by restriction mapping and nucleotide sequencing.

[0287] Recombinant bacteria from one clone were induced to express the SOD-NANB<sub>81</sub> polypeptide by growing the bacteria in the presence of IPTG.

#### IV.B.3. Identification of the Polypeptide Encoded Within Clone 5-1-1 as an HCV and NANBH Associated Antigen.

[0288] The polypeptide encoded within the HCV cDNA of clone 5-1-1 was identified as a NANBH associated antigen by demonstrating that sera of chimpanzees and humans infected with NANBH reacted immunologically with the fusion polypeptide, SOD-NANB<sub>5-1-1</sub>, which is comprised of superoxide dismutase at its N-terminus and the in-frame 5-1-1 antigen at its C-terminus. This was accomplished by "Western" blotting (Towbin et al. (1979)) as follows.

[0289] A recombinant strain of bacteria transformed with an expression vector encoding the SOD-NANB<sub>5-1-1</sub> polypeptide, described in Section IV.B.I., was induced to express the fusion polypeptide by growth in the presence of IPTG. Total bacterial lysate was subjected to electrophoresis through polyacrylamide gels in the presence of SDS according to Laemmli (1970). The separated polypeptides were transferred onto nitrocellulose filters (Towbin et al. (1979)). The filters were then cut into thin strips, and the strips were incubated individually with the different chimpanzee and human sera. Bound antibodies were detected by further incubation with <sup>125</sup>I-labeled sheep anti-human Ig, as described in Section IV.A.1.

[0290] The characterization of the chimpanzee sera used for the Western blots and the results, shown in the photograph of the autoradiographed strips, are presented in Fig. 33. Nitrocellulose strips containing polypeptides were incubated with sera derived from chimpanzees at different times during acute NANBH (Hutchinson strain) infections (lanes 1-16), hepatitis A infections (lanes 17-24, and 26-33), and hepatitis B infections (lanes 34-44). Lanes 25 and 45 show positive controls in which the immunoblots were incubated with serum from the patient used to identify the recombinant clone 5-1-1 in the original screening of the lambda-gt11 cDNA library (see Section IV.A.1.).

[0291] The band visible in the control lanes, 25 and 45, in Fig. 23 reflects the binding of antibodies to the NANB<sub>5-1-1</sub> moiety of the SOD fusion polypeptide. These antibodies do not exhibit binding to SOD alone, since this has also been included as a negative control in these samples, and would have appeared as a band migrating significantly faster than the SOD-NANB<sub>5-1-1</sub> fusion polypeptide.

[0292] Lanes 1-16 of Fig. 33 show the binding of antibodies in sera samples of 4 chimpanzees; the sera were obtained just prior to infection with NANBH, and sequentially during acute infection. As seen from the figure, whereas antibodies which reacted immunologically with the SOD-NANB<sub>5-1-1</sub> polypeptide were absent in sera samples obtained before administration of infectious HCV inoculum and during the early acute phase of infection, all 4 animals eventually induced circulating antibodies to this polypeptide during the late part of, or following the acute phase. Additional bands observed on the immunoblots in the cases of chimps numbers 3 and 4 were due to background binding to host bacterial proteins.

[0293] In contrast to the results obtained with sera from chimps infected with NANBH, the development of antibodies to the NANB<sub>5-1-1</sub> moiety of the fusion polypeptide was not observed in 4 chimpanzees infected with HAV or 3 chimpanzees infected with HBV. The only binding in these cases was background binding to the host bacterial proteins, which also occurred in the HCV infected samples.

[0294] The characterization of the human sera used for the Western blots, and the results, which are shown in the photograph of the autoradiographed strips, are presented in Fig. 34. Nitrocellulose strips containing polypeptides were incubated with sera derived from humans at different times during infections with NANBH (lanes 1-21), HAV (lanes 33-40), and HBV (lanes 41-49). Lanes 25 and 50 show positive controls in which the immunoblots were incubated with serum from patient used in the original screening of the lambda-gt11 library, described supra. Lanes 22-24 and 26-32 show "non-infected" controls in which the sera was from "normal" blood donors.

[0295] As seen in Fig. 34, sera from nine NANBH patients, including the serum used for screening the lambda-gt11 library, contained antibodies to the NANB<sub>5-1-1</sub> moiety of the fusion polypeptide. Sera from three patients with NANBH did not contain these antibodies. It is possible that the anti-NANB<sub>5-1-1</sub> antibodies will develop at a future date in these patients. It is also possible that this lack of reaction resulted from a different NANBV agent being causative of the disease in the individuals from which the non-responding serum was taken.

[0296] Fig. 34 also shows that sera from many patients infected with HAV and HBV did not contain anti-NANB<sub>5-1-1</sub> antibodies, and that these antibodies were also not present in the sera from "normal" controls. Although one HAV patient (lane 36) appears to contain anti-NANB<sub>5-1-1</sub> antibodies, it is possible that this patient had been previously infected with HCV, since the incidence of NANBH is very high and since it is often subclinical.

[0297] These serological studies indicate that the cDNA in clone 5-1-1 encodes epitopes which are recognized specifically by sera from patients and animals infected with BB-NANBV. In addition, the cDNA does not appear to be derived from the primate genome. A hybridization probe made from clone 5-1-1 or from clone 81 did not hybridize to "Southern" blots of control human and chimpanzee genomic DNA from uninfected individuals under conditions where unique, single-copy genes are detectable. These probes also did not hybridize to Southern blots of control bovine

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genomic DNA.

#### IV.B.4. Expression of the Polypeptide Encoded In a Composite of the HCV cDNAs in Clones 36, 81 and 32

5 [0298] The HCV polypeptide which is encoded in the ORF which extends through clones 36, 81 and 32 was expressed as a fusion polypeptide with SOD. This was accomplished by inserting the composite cDNA, C100, into an expression cassette which contains the human superoxide dismutase gene, inserting the expression cassette into a yeast expression vector, and expressing the polypeptide in yeast.

10 [0299] An expression cassette containing the composite C100 cDNA derived from clones 36, 81, and 32, was constructed by inserting the ~1270bp EcoRI fragment into the EcoRI site of the vector pS3-56 (also called pS356), yielding the plasmid pS3-56<sub>C100</sub>. The construction of C100 is described in Section IV.A.16, *supra*.

15 [0300] The vector pS3-56, which is a pBR322 derivative, contains an expression cassette which is comprised of the ADH2/GAPDH hybrid yeast promoter upstream of the human superoxide dismutase gene, and a downstream GAPDH transcription terminator. A similar cassette, which contains these control elements and the superoxide dismutase gene has been described in Cousens et al. (1987), and in copending application EPO 196,056, published October 1, 1986, which is commonly owned by the herein assignee. The cassette in pS3-56, however, differs from that in Cousens et al. (1987) in that the heterologous proinsulin gene and the immunoglobulin hinge are deleted, and in that the gln<sub>154</sub> of the superoxide dismutase is followed by an adaptor sequence which contains an EcoRI site. The sequence of the adaptor is:

20

5'-AAT TTG GGA ATT CCA TAA TGA G        -3'  
AC CCT TAA GGT ATT ACT CAG CT

25

The EcoRI site allows the insertion of heterologous sequences which, when expressed from a vector containing the cassette, yield polypeptides which are fused to superoxide dismutase via an oligopeptide linker containing the amino acid sequence:

30

-asn-leu-gly-ile-arg-

35 [0301] A sample of pS356 has been deposited on 29 April 1988 under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville, Maryland 20853, and has been assigned Accession No. 67683. The terms and conditions for availability and access to the deposit, and for maintenance of the deposit are the same as those specified in Section II.A., for strains containing NANBV-cDNAs. This deposit is intended for convenience only, and is not required to practice the present invention in view of the description here. The deposited material is hereby incorporated herein by reference.

40 [0302] After recombinants containing the C100 cDNA insert in the correct orientation were isolated, the expression cassette containing the C100 cDNA was excised from pS3-56<sub>C100</sub> with BamHI, and a fragment of ~3400bp which contains the cassette was isolated and purified. This fragment was then inserted into the BamHI site of the yeast vector pAB24.

45 [0303] Plasmid pAB24, the significant features of which are shown in Fig. 35, is a yeast shuttle vector which contains the complete 2 micron sequence for replication [Broach (1981)] and pBR322 sequences. It also contains the yeast URA3 gene derived from plasmid YEp24 [Botstein et al. (1979)], and the yeast LEU<sup>2d</sup> gene derived from plasmid pC1/1. EPO Pub. No. 116,201. Plasmid pAB24 was constructed by digesting YEp24 with EcoRI and religating the vector to remove the partial 2 micron sequences. The resulting plasmid, YEP24deltaRI, was linearized by digestion with ClaI and ligated with the complete 2 micron plasmid which had been linearized with ClaI. The resulting plasmid, pCBou, was then digested with XbaI and the 8605 bp vector fragment was gel isolated. This isolated XbaI fragment was ligated with a 4460 bp XbaI fragment containing the LEU<sup>2d</sup> gene isolated from pC1/1; the orientation of the LEU<sup>2d</sup> gene is in the same direction as the URA3 gene. Insertion of the expression was in the unique BamHI site of the pBR322 sequence, thus interrupting the gene for bacterial resistance to tetracycline.

50 [0304] The recombinant plasmid which contained the SOD-C100 expression cassette, pAB24C100-3, was transformed into yeast strain JSC 308, as well as into other yeast strains. The cells were transformed as described by Hinnen et al. (1978), and plated onto ura-selective plates. Single colonies were inoculated into leu-selective media and grown to saturation. The culture was induced to express the SOD-C100 polypeptide (called C100-3) by growth in YEP containing 1% glucose.

[0305] Strain JSC 308 is of the genotype MAT @, leu2, ura3(del) DM15 (GAP/ADR1) Integrated at the ADR1 locus.

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In JSC 308, over-expression of the positive activator gene product, ADR1, results in hyperderepression (relative to an ADR1 wild type control) and significantly higher yields of expressed heterologous proteins when such proteins are synthesized via an ADH2 UAS regulatory system. A sample of JSC 308 has been deposited on 5 May 1988 with the ATCC under the conditions of the Budapest Treaty, and has been assigned Accession No. 20879. The terms and conditions for availability and access to the deposit, and for maintenance of the deposit are the same as those specified in Section II.A., for strains containing HCV cDNAs.

[0306] The complete C100-3 fusion polypeptide encoded in pAB24C100-3 should contain 154 amino acids of human SOD at the amino-terminus, 5 amino acid residues derived from the synthetic adaptor containing the EcoRI site, 363 amino acid residues derived from C100 cDNA, and 5 carboxy-terminal amino acids derived from the MS2 nucleotide sequence adjoining the HCV CDNA sequence in clone 32. (See Section IV.A.7.) The putative amino acid sequence of the carboxy-terminus of this polypeptide, beginning at the penultimate Ala residue of SOD, is shown in Fig. 36; also shown is the nucleotide sequence encoding this portion of the polypeptide.

#### IV.B.5. Identification of the Polypeptide Encoded within C100 as an NANBH Associated Antigen

[0307] The C100-3 fusion polypeptide expressed from plasmid pAB24C100-3 in yeast strain JSC 308 was characterized with respect to size, and the polypeptide encoded within C100 was identified as an NANBH-associated antigen by its immunological reactivity with serum from a human with chronic NANBH.

[0308] The C100-3 polypeptide, which was expressed as described in Section IV.B.4., was analyzed as follows. Yeast JSC 308 cells were transformed with pAB24, or with pAB24C100-3, and were induced to express the heterologous plasmid encoded polypeptide. The induced yeast cells in 1 ml of culture ( $OD_{650\text{ nm}} \sim 20$ ) were pelleted by centrifugation at 10,000 rpm for 1 minute, and were lysed by vortexing them vigorously ( $10 \times 1 \text{ min}$ ) with 2 volumes of solution and 1 volume of glass beads (0.2 millimicron diameter). The solution contained 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM phenylmethylsulphonyl fluoride (PMSF), and 1 microgram/ml pepstatin. Insoluble material in the lysate, which includes the C100-3 polypeptide, was collected by centrifugation (10,000 rpm for 5 minutes), and was dissolved by boiling for 5 minutes in Laemmli SDS sample buffer. [See Laemmli (1970)]. An amount of polypeptides equivalent to that in 0.3 ml of the induced yeast culture was subjected to electrophoresis through 10% polyacrylamide gels in the presence of SDS according to Laemmli (1970). Protein standards were co-electrophoresed on the gels. Gels containing the expressed polypeptides were either stained with Coomassie brilliant blue, or were subjected to "Western" blotting as described in Section IV.B.2., using serum from a patient with chronic NANBH to determine the immunological reactivity of the polypeptides expressed from pAB24 and from pAB24C100-3.

[0309] The results are shown in Fig. 37. In Fig. 37A the polypeptides were stained with Coomassie brilliant blue. The insoluble polypeptide(s) from JSC 308 transformed with pAB24 and from two different colonies of JSC transformed with pAB24C100-3 are shown in lane 1 (pAB24), and lanes 2 and 3, respectively. A comparison of lanes 2 and 3 with lane 1 shows the induced expression of a polypeptide corresponding to a molecular weight of ~54,000 daltons from JSC 308 transformed with pAB24C100-3, which is not induced in JSC 308 transformed with pAB24. This polypeptide is indicated by the arrow.

[0310] Fig. 37B shows the results of the Western blots of the insoluble polypeptides expressed in JSC 308 transformed with pAB24 (lane 1), or with pAB24C100-3 (lane 2). The polypeptides expressed from pAB24 were not immunologically reactive with serum from a human with NANBH. However, as indicated by the arrow, JSC 308 transformed with pAB24C100-3 expressed a polypeptide of ~54,000 dalton molecular weight which did react immunologically with the human NANBH serum. The other immunologically reactive polypeptides in lane 2 may be degradation and/or aggregation products of this ~54,000 dalton polypeptide.

#### IV.B.6. Purification of Fusion Polypeptide C100-3

[0311] The fusion polypeptide, C100-3, comprised of SOD at the N-terminus and in-frame C100 HCV-polypeptide at the C-terminus was purified by differential extraction of the insoluble fraction of the extracted host yeast cells in which the polypeptide was expressed.

[0312] The fusion polypeptide, C100-3, was expressed in yeast strain JSC 308 transformed with pAB24C100-3, as described in Section IV.B.4. The yeast cells were then lysed by homogenization, the insoluble material in the lysate was extracted at pH 12.0, and C100-3 in the remaining insoluble fraction was solubilized in buffer containing SDS.

[0313] The yeast lysate was prepared essentially according to Nagahuma et al. (1984). A yeast cell suspension was prepared which was 33% cells (v/v) suspended in a solution (Buffer A) containing 20 mM Tris HCl, pH 8.0, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride (PMSF). An aliquot of the suspension (15 ml) was mixed with an equal volume of glass beads (0.45-0.50 mm diameter), and the mixture was vortexed at top speed on a Super Mixer (Lab Line Instruments, Inc.) for 8 min. The homogenate and glass beads were separated, and the glass beads were washed 3 times with the same volume of Buffer A as the original packed cells. After combining the washes and ho-

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mogenate, the insoluble material in the lysate was obtained by centrifuging the homogenate at 7,000 x g for 15 minutes at 4°C, resuspending the pellets in Buffer A equal to twice the volume of original packed cells, and re-pelleting the material by centrifugation at 7,000 x g for 15 min. This washing procedure was repeated 3 times.

5 [0314] The insoluble material from the lysate was extracted at pH 12.0 as follows. The pellet was suspended in buffer containing 0.5 M NaCl, 1 mM EDTA, where the suspending volume was equal to 1.8 times the of the original packed cells. The pH of the suspension was adjusted by adding 0.2 volumes of 0.4 M Na phosphate buffer, pH 12.0. After mixing, the suspension was centrifuged at 7,000 x g for 15 min at 4°C, and the supernatant removed. The extraction was repeated 2 times. The extracted pellets were washed by suspending them in 0.5 M NaCl, 1 mM EDTA, using a suspension volume equal to two volumes of the original packed cells, followed by centrifugation at 7,000 x g for 15

10 min at 4°C. [0315] The C100-3 polypeptide in the extracted pellet was solubilized by treatment with SDS. The pellets were suspended in Buffer A equal to 0.9 volumes of the original packed cell volume, and 0.1 volumes of 2% SDS was added. After the suspension was mixed, it was centrifuged at 7,000 x g for 15 min at 4°C. The resulting pellet was extracted 3 more times with SDS. The resulting supernatants, which contained C100-3 were pooled.

15 [0316] This procedure purifies C100-3 more than 10-fold from the insoluble fraction of the yeast homogenate, and the recovery of the polypeptide is greater than 50%.

[0317] The purified preparation of fuslon polypeptide was analyzed by polyacrylamide gel electrophoresis according to Laemmli (1970). Based upon this analysis, the polypeptide was greater than 80% pure, and had an apparent molecular weight of ~54,000 daltons.

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#### IV.C. Identification of RNA in Infected Individuals Which Hybridizes to HCV cDNA.

##### IV.C.1. Identification of RNA in the Liver of a Chimpanzee With NANBH Which Hybridizes to HCV cDNA.

25 [0318] RNA from the liver of a chimpanzee which had NANBH was shown to contain a species of RNA which hybridized to the HCV cDNA contained within clone 81 by Northern blotting, as follows.

[0319] RNA was isolated from a liver biopsy of the chimpanzee from which the high titer plasma was derived (see Section IV.A.1.) using techniques described in Maniatis et al. (1982) for the isolation of total RNA from mammalian cells, and for its separation into poly A<sup>+</sup> and poly A<sup>-</sup> fractions. These RNA fractions were subjected to electrophoresis on a formaldehyde/agarose gel (1% w/v), and transferred to nitrocellulose. (Maniatis et al. (1982)). The nitrocellulose filters were hybridized with radiolabeled HCV CDNA from clone 81 (see Fig. 4 for the nucleotide sequence of the insert.) To prepare the radiolabeled probe, the HCV cDNA insert isolated from clone 81 was radiolabeled with <sup>32</sup>P by nick translation using DNA Polymerase I (Maniatis et al. (1982)). Hybridization was for 18 hours at 42°C in a solution containing 10% (w/v) Dextran sulphate, 50% (w/v) deionized formamide, 750 mM NaCl, 75 mM Na citrate, 20 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 6.5, 0.1% SDS, 0.02% (w/v) bovine serum albumin (BSA), 0.02% (w/v) Ficoll-400, 0.02% (w/v) polyvinylpyrrolidone, 100 micrograms/ml salmon sperm DNA which had been sheared by sonication and denatured, and 10<sup>6</sup> CPM/ml of the nick-translated CDNA probe.

[0320] An autoradiograph of the probed filter is shown in Fig. 38. Lane 1 contains <sup>32</sup>P-labeled restriction fragment markers. Lanes 2-4 contain chimpanzee liver RNA as follows: lane 2 contains 30 micrograms of total RNA; lane 3 contains 30 micrograms of poly A<sup>-</sup> RNA; and lane 4 contains 20 micrograms of poly A<sup>+</sup> RNA. As shown in Fig. 38, the liver of the chimpanzee with NANBH contains a heterogeneous population of related poly A<sup>+</sup> RNA molecules which hybridizes to the HCV CDNA probe, and which appears to be from about 5000 nucleotides to about 11,000 nucleotides in size. This RNA, which hybridizes to the HCV cDNA, could represent viral genomes and/or specific transcripts of the viral genome.

45 [0321] The experiment described in Section IV.C.2., infra, is consistent with the suggestion that HCV contains an RNA genome.

##### IV.C.2. Identification of HCV Derived RNA In Serum from Infected Individuals.

50 [0322] Nucleic acids were extracted from particles isolated from high titer chimpanzee NANBH plasma as described in Section IV.A.1.. Aliquots (equivalent to 1 ml of original plasma) of the isolated nucleic acids were resuspended in 20 microliters 50 mM Hepes, pH 7.5, 1 mM EDTA and 16 micrograms/ml yeast soluble RNA. The samples were denatured by boiling for 5 minutes followed by immediate freezing, and were treated with RNase A (5 microliters containing 0.1 mg/ml RNase A in 25 mM EDTA, 40 mM Hepes, pH 7.5) or with DNase I (5 microliters containing 1 unit DNase I in 10 mM MgCl<sub>2</sub>, 25 mM Hepes, pH 7.5); control samples were incubated without enzyme. Following incubation, 230 microliters of ice-cold 2XSSC containing 2 micrograms/ml yeast soluble RNA was added, and the samples were filtered on a nitrocellulose filter. The filters were hybridized with a cDNA probe from clone 81, which had been <sup>32</sup>P-labeled by nick-translation. Fig. 39 shows an autoradiograph of the filter. Hybridization signals were detected in the DNase treated

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and control samples (lanes 2 and 1, respectively), but were not detected in the RNase treated sample (lane 3). Thus, since RNase A treatment destroyed the nucleic acids isolated from the particles, and DNase I treatment had no effect, the evidence strongly suggests that the HCV genome is composed of RNA.

5 IV.C.3. Detection of Amplified HCV Nucleic Acid Sequences derived from HCV Nucleic Acid Sequences in Liver and Plasma Specimens from Chimpanzees with NANBH

[0323] HCV nucleic acids present in liver and plasma of chimpanzees with NANBH, and in control chimpanzees, were amplified using essentially the polymerase chain reaction (PCR) technique described by Saiki et al. (1986). The primer oligonucleotides were derived from the HCV cDNA sequences in clone 81, or clones 36 and 37. The amplified sequences were detected by gel electrophoresis and Southern blotting, using as probes the appropriate cDNA oligomer with a sequence from the region between, but not including, the two primers.

[0324] Samples of RNA containing HCV sequences to be examined by the amplification system were isolated from liver biopsies of three chimpanzees with NANBH, and from two control chimpanzees. The isolation of the RNA fraction was by the guanidinium thiocyanate procedure described in Section IV.C.1.

[0325] Samples of RNA which were to be examined by the amplification system were also isolated from the plasmas of two chimpanzees with NANBH, and from one control chimpanzee, as well as from a pool of plasmas from control chimpanzees. One infected chimpanzee had a CID/ml equal to or greater than  $10^6$ , and the other infected chimpanzee had a CID/ml equal to or greater than  $10^5$ .

[0326] The nucleic acids were extracted from the plasma as follows. Either 0.1 ml or 0.01 ml of plasma was diluted to a final volume of 1.0 ml, with a TENB/proteinase K/SDS solution (0.05 M Tris-HCl, pH 8.0, 0.001 M EDTA, 0.1 M NaCl, 1 mg/ml Proteinase K, and 0.5% SDS) containing 10 micrograms/ml polyadenylic acid, and incubated at 37°C for 60 minutes. After this proteinase K digestion, the resultant plasma fractions were deproteinized by extraction with TE (10.0 mM Tris-HCl, pH 8.0, 1 mM EDTA) saturated phenol. The phenol phase was separated by centrifugation, and was reextracted with TENB containing 0.1% SDS. The resulting aqueous phases from each extraction were pooled, and extracted twice with an equal volume of phenol/chloroform/isoamyl alcohol [1:1(99:2)], and then twice with an equal volume of a 99:1 mixture of chloroform/isoamyl alcohol. Following phase separation by centrifugation, the aqueous phase was brought to a final concentration of 0.2 M Na Acetate, and the nucleic acids were precipitated by the addition of two volumes of ethanol. The precipitated nucleic acids were recovered by ultracentrifugation in a SW 41 rotor at 38 K, for 60 minutes at 4 ° C.

[0327] In addition to the above, the high titer chimpanzee plasma and the pooled control plasma alternatively were extracted with 50 micrograms of poly A carrier by the procedure of Chomczynski and Sacchi (1987). This procedure uses an acid guanidinium thiocyanate extraction. RNA was recovered by centrifugation at 10,000 RPM for 10 minutes at 4°C in an Eppendorf microfuge.

[0328] On two occasions, prior to the synthesis of cDNA in the PCR reaction, the nucleic acids extracted from plasma by the proteinase K/SDS/phenol method were further purified by binding to and elution from S and S Elutip-R Columns. The procedure followed was according to the manufacturer's directions.

[0329] The cDNA used as a template for the PCR reaction was derived from the nucleic acids (either total nucleic acids or RNA) prepared as described above. Following ethanol precipitation, the precipitated nucleic acids were dried, and resuspended in DEPC treated distilled water. Secondary structures in the nucleic acids were disrupted by heating at 65°C for 10 minutes, and the samples were immediately cooled on ice. cDNA was synthesized using 1 to 3 micrograms of total chimpanzee RNA from liver, or from nucleic acids (or RNA) extracted from 10 to 100 microliters of plasma. The synthesis utilized reverse transcriptase, and was in a 25 microliter reaction, using the protocol specified by the manufacturer, BRL. The primers for cDNA synthesis were those also utilized in the PCR reaction, described below. All reaction mixtures for cDNA synthesis contained 23 units of the RNAase inhibitor, RNASIN (Fisher/Promega). Following cDNA synthesis, the reaction mixtures were diluted with water, boiled for 10 minutes, and quickly chilled on ice.

[0330] The PCR reactions were performed essentially according to the manufacturer's directions (Cetus-Perkin-Elmer), except for the addition of 1 microgram of RNase A. The reactions were carried out in a final volume of 100 microliters. The PCR was performed for 35 cycles, utilizing a regimen of 37°C, 72°C, and 94°C.

[0331] The primers for cDNA synthesis and for the PCR reactions were derived from the HCV cDNA sequences in either clone 81, clone 36, or clone 37b. (The HCV cDNA sequences of clones 81, 36, and 37b are shown in Figs. 4, 5, and 10, respectively.) The sequences of the two 16-mer primers derived from clone 81 were:

5' CAA TCA TAC CTG ACA G 3'

and

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5' GAT AAC CTC TGC CTG A 3'.

The sequence of the primer from clone 36 was:

5' GCA TGT CAT GAT GTA T 3'.

The sequence of the primer from clone 37b was:

5' ACA ATA CGT GTG TCA C 3'.

In the PCR reactions, the primer pairs consisted of either the two 16-mers derived from clone 81, or the 16-mer from clone 36 and the 16-mer from clone 37b.

[0332] The PCR reaction products were analyzed by separation of the products by alkaline gel electrophoresis, followed by Southern blotting, and detection of the amplified HCV-cDNA sequences with a <sup>32</sup>P-labeled internal oligo-nucleotide probe derived from a region of the HCV cDNA which does not overlap the primers. The PCR reaction mixtures were extracted with phenol/chloroform, and the nucleic acids precipitated from the aqueous phase with salt and ethanol. The precipitated nucleic acids were collected by centrifugation, and dissolved in distilled water. Aliquots of the samples were subjected to electrophoresis on 1.8% alkaline agarose gels. Single stranded DNA of 60, 108, and 161 nucleotide lengths were co-electrophoresed on the gels as molecular weight markers. After electrophoresis, the DNAs in the gel were transferred onto Biorad Zeta Probe™ paper. Prehybridization and hybridization, and wash conditions were those specified by the manufacturer (Biorad).

[0333] The probes used for the hybridization-detection of amplified HCV cDNA sequences were the following. When the pair of PCR primers were derived from clone 81, the probe was an 108-mer with a sequence corresponding to that which is located in the region between the sequences of the two primers. When the pair of PCR primers were derived from clones 36 and 37b, the probe was the nick-translated HCV cDNA insert derived from clone 35. The primers are derived from nucleotides 155-170 of the clone 37b insert, and 206-268 of the clone 36 insert. The 3'-end of the HCV cDNA insert in clone 35 overlaps nucleotides 1-186 of the insert in clone 36; and the 5'-end of clone 35 insert overlaps nucleotides 207-269 of the insert in clone 37b. (Compare Figs. 5, 8 and 10.) Thus, the cDNA insert in clone 35 spans part of the region between the sequences of the clone 36 and 37b derived primers, and is useful as a probe for the amplified sequences which include these primers.

[0334] Analysis of the RNA from the liver specimens was according to the above procedure utilizing both sets of primers and probes. The RNA from the liver of the three chimpanzees with NANBH yielded positive hybridization results for amplification sequences of the expected size (161 and 586 nucleotides for 81 and 36 and 37b, respectively), while the control chimpanzees yielded negative hybridization results. The same results were achieved when the experiment was repeated three times.

[0335] Analysis of the nucleic acids and RNA from plasma was also according to the above procedure utilizing the primers and probe from clone 81. The plasmas were from two chimpanzees with NANBH, from a control chimpanzee, and pooled plasmas from control chimpanzees. Both of the NANBH plasmas contained nucleic acids/RNA which yielded positive results in the PCR amplified assay, while both of the control plasmas yielded negative results. These results have been repeatably obtained several times.

#### IV.D. Radioimmunoassay for Detecting HCV Antibodies in Serum from Infected Individuals

[0336] Solid phase radioimmunoassays to detect antibodies to HCV antigens were developed based upon Tsu and Herzenberg (1980). Microtiter plates (Immulon 2, Removawell strips) are coated with purified polypeptides containing HCV epitopes. The coated plates are incubated with either human serum samples suspected of containing antibodies to the HCV epitopes, or to appropriate controls. During incubation, antibody, if present, is immunologically bound to the solid phase antigen. After removal of the unbound material and washing of the microtiter plates, complexes of human antibody-NANBV antigen are detected by incubation with <sup>125</sup>I-labeled sheep anti-human immunoglobulin. Unbound labeled antibody is removed by aspiration, and the plates are washed. The radioactivity in individual wells is determined; the amount of bound human anti-HCV antibody is proportional to the radioactivity in the well.

#### IV.D.1. Purification of Fusion Polypeptide SOD-NANB<sub>5-1-1</sub>

[0337] The fusion polypeptide SOD-NANB<sub>5-1-1</sub>, expressed in recombinant bacteria as described in Section IV.B.1., was purified from the recombinant *E. coli* by differential extraction of the cell extracts with urea, followed by chromatography on anion and cation exchange columns as follows.

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[0338] Thawed cells from 1 liter of culture were resuspended in 10 ml of 20% (w/v) sucrose containing 0.01M Tris HCl, pH 8.0, and 0.4 ml of 0.5M EDTA, pH 8.0 was added. After 5 minutes at 0°C, the mixture was centrifuged at 4,000 x g for 10 minutes. The resulting pellet was suspended in 10 ml of 25% (w/v) sucrose containing 0.05 M Tris HCl, pH 8.0, 1 mM phenylmethylsulfonylfluoride (PMSF) and 1 microgram/ml pepstatin A, followed by addition of 0.5 ml lys-  
 5 ozyme (10 mg/ml) and incubation at 0°C for 10 minutes. After the addition of 10 ml 1% (v/v) Triton X-100 in 0.05 M Tris HCl, pH 8.0, 1 mM EDTA, the mixture was incubated an additional 10 min at 0°C with occasional shaking. The resulting viscous solution was homogenized by passage 6 times through a sterile 20-gauge hypodermic needle, and centrifuged at 13,000 x g for 25 minutes. The pelleted material was suspended in 5 ml of 0.01 M Tris HCl pH 8.0, and the suspension centrifuged at 4,000 x g for 10 minutes. The pellet, which contained SOD-NANB<sub>5-1-1</sub> fusion protein,  
 10 was dissolved in 5 ml of 6 M urea in 0.02 M Tris HCl, pH 8.0, 1 mM dithiothreitol (Buffer A), and was applied to a column of Q-Sepharose Fast Flow equilibrated with Buffer A. Polypeptides were eluted with a linear gradient of 0.0 to 0.3 M NaCl in Buffer A. After elution, fractions were analyzed by polyacrylamide gel electrophoresis in the presence of SDS to determine their content of SOD-NANB<sub>5-1-1</sub>. Fractions containing this polypeptide were pooled, and dialyzed against 6 M urea in 0.02 M sodium phosphate buffer, pH 6.0, 1 mM dithiothreitol (Buffer B). The dialyzed sample was applied  
 15 on a column of S-Sepharose Fast Flow equilibrated with Buffer B, and polypeptides eluted with a linear gradient of 0.0 to 0.3 M NaCl in Buffer B. The fractions were analyzed by polyacrylamide gel electrophoresis for the presence of SOD-NANB<sub>5-1-1</sub>, and the appropriate fractions were pooled.

[0339] The final preparation of SOD-NANB<sub>5-1-1</sub> polypeptide was examined by electrophoresis on polyacrylamide gels in the presence of SDS. Based upon this analysis, the preparation was more than 80% pure.

#### IV.D.2. Purification of Fusion Polypeptide SOD-NANB<sub>81</sub>.

[0340] The fusion polypeptide SOD-NANB<sub>81</sub>, expressed in recombinant bacteria as described in Section IV.B.2., was purified from recombinant *E. coli* by differential extraction of the cell extracts with urea, followed by chromatography  
 25 on anion and cation exchange columns utilizing the procedure described for the isolation of fusion polypeptide SOD-NANB<sub>5-1-1</sub> (See Section IV.D.1.).

[0341] The final preparation of SOD-NANB<sub>81</sub> polypeptide was examined by electrophoresis on polyacrylamide gels in the presence of SDS. Based upon this analysis, the preparation was more than 50% pure.

#### IV.D.3. Detection of Antibodies to HCV Epitopes by Solid Phase Radioimmunoassay.

[0342] Serum samples from 32 patients who were diagnosed as having NANBH were analyzed by radioimmunoassay (RIA) to determine whether antibodies to HCV epitopes present in fusion polypeptides SOD-NANB<sub>5-1-1</sub> and SOD-NANB<sub>81</sub> were detected.

[0343] Microtiter plates were coated with SOD-NANB<sub>5-1-1</sub> or SOD-NANB<sub>81</sub>, which had been partially purified according to Sections IV.D.1. and IV.D.2., respectively. The assays were conducted as follows.

[0344] One hundred microliter aliquots containing 0.1 to 0.5 micrograms of SOD-NANB<sub>5-1-1</sub> or SOD-NANB<sub>81</sub> in 0.125 M Na borate buffer, pH 8.3, 0.075 M NaCl (BBS) was added to each well of a microtiter plate (Dynatech Immulon 2 Removawell Strips). The plate was incubated at 4°C overnight in a humid chamber, after which, the protein solution  
 40 was removed and the wells washed 3 times with BBS containing 0.02% Triton X-100 (BBST). To prevent non-specific binding, the wells were coated with bovine serum albumin (BSA) by addition of 100 microliters of a 5 mg/ml solution of BSA in BBS followed by incubation at room temperature for 1 hour; after this incubation the BSA solution was removed. The polypeptides in the coated wells were reacted with serum by adding 100 microliters of serum samples  
 45 diluted 1:100 in 0.01M Na phosphate buffer, pH 7.2, 0.15 M NaCl (PBS) containing 10 mg/ml BSA, and incubating the serum containing wells for 1 hr at 37°C. After incubation, the serum samples were removed by aspiration, and the wells were washed 5 times with BBST. Anti-NANB<sub>5-1-1</sub> and Anti-NANB<sub>81</sub> bound to the fusion polypeptides was determined by the binding of <sup>125</sup>I-labeled F(ab)<sub>2</sub> sheep anti-human IgG to the coated wells. Aliquots of 100 microliters of the labeled probe (specific activity 5-20 microcuries/microgram) were added to each well, and the plates were incubated  
 50 at 37°C for 1 hour, followed by removal of excess probe by aspiration, and 5 washes with BBST. The amount of radioactivity bound in each well was determined by counting in a counter which detects gamma radiation.

[0345] The results of the detection of anti-NANB<sub>5-1-1</sub> and anti-NANB<sub>81</sub> in individuals with NANBH is presented in Table 1.

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Table 1

Detection of Anti-5-1-1 and Anti-81 in Sera of NANB, HAV and HBV Hepatitis Patients			
Patient Reference Number	Diagnosis	S/N	
		Anti-5-1-1	Anti-81
1. 28 <sup>1</sup>	Chronic NANB, IVD <sup>2</sup>	0.77	4.20
	Chronic NANB, IVD	1.14	5.14
	Chronic NANB, IVD	2.11	4.05
2. 29 <sup>1</sup>	AVH <sup>3</sup> , NANB, Sporadic	1.09	1.05
	Chronic, NANB	33.89	11.39
	Chronic, NANB	36.22	13.67
3. 30 <sup>1</sup>	AVH, NANB, IVD	1.90	1.54
	Chronic NANB, IVD	34.17	30.28
	Chronic NANB, IVD	32.45	30.84
4. 31	Chronic NANB, PT <sup>4</sup>	16.09	8.05
5. 32 <sup>1</sup>	Late AVH NANB, IVD	0.69	0.94
	Late AVH NANB, IVD	0.73	0.68
6. 33 <sup>1</sup>	AVH, NANB, IVD	1.66	1.96
	AVH, NANB, IVD	1.53	0.56
7. 34 <sup>1</sup>	Chronic NANB, PT	34.40	7.55
	Chronic NANB, PT	45.55	13.11
	Chronic NANB, PT	41.58	13.45
	Chronic NANB, PT	44.20	15.48
8. 35 <sup>1</sup>	AVH NANB, IVD	31.92	31.95
	"Healed" recent NANB, AVH	6.87	4.45
9. 36	Late AVH NANB PT	11.84	5.79
10. 37	AVH NANB, IVD	6.52	1.33
11. 38	Late AVH NANB, PT	39.44	39.18
12. 39	Chronic NANB, PT	42.22	37.54
13. 40	AVH, NANB, PT	1.35	1.17
14. 41	Chronic NANB? PT	0.35	0.28
15. 42	AVH, NANB, IVD	6.25	2.34
16. 43	Chronic NANB, PT	0.74	0.61
17. 44	AVH, NANB, PT	5.40	1.83
18. 45	Chronic, NANB, PT	0.52	0.32
19. 46	AVH, NANB	23.35	4.45
20. 47	AVH, Type A	1.60	1.35
21. 48	AVH, Type A	1.30	0.66
22. 49	AVH, Type A	1.44	0.74
23. 50	Resolved Recent AVH, Type A	0.48	0.56
24. 51	AVH, Type A	0.68	0.64
	Resolved AVH, Type A	0.80	0.65
25. 52	Resolved Recent AVH, Type A	1.38	1.04
	Resolved Recent AVH, Type A	0.80	0.65
26. 53	AVH, Type A	1.85	1.16
	Resolved Recent AVH, Type A	1.02	0.88
27. 54	AVH, Type A	1.35	0.74
28. 55	Late AVH, HBV	0.58	0.55

<sup>1</sup>Sequential serum samples available from these patients<sup>2</sup>IVD=Intravenous Drug User<sup>3</sup>AVH=Acute viral hepatitis<sup>4</sup>PT=Post transfusion

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Table 1 (continued)

Detection of Anti-5-1-1 and Anti-81 in Sera of NANB, HAV and HBV Hepatitis Patients			
Patient Reference Number	Diagnosis	S/N	
		Anti-5-1-1	Anti-81
29. 56	Chronic HBV	0.84	1.06
30. 57	Late AVH, HBV	3.20	1.60
31. 58	Chronic HBV	0.47	0.46
32. 59 <sup>1</sup>	AVH, HBV	0.73	0.60
	Kealed AVH, HBV	0.43	0.44
33. 60 <sup>1</sup>	AVH, HBV	1.06	0.92
	Healed AVH, HBV	0.75	0.68
34. 61 <sup>1</sup>	AVH, HBV	1.66	0.61
	Healed AVH, HBV	0.63	0.36
35. 62 <sup>1</sup>	AVH, HBV	1.02	0.73
	Healed AVH, HBV	0.41	0.42
36. 63 <sup>1</sup>	AVH, HBV	1.24	1.31
	Healed AVH, HBV	1.55	0.45
37. 64 <sup>1</sup>	AVH, HBV	0.82	0.79
	Healed AVH, HBV	0.53	0.37
38. 65 <sup>1</sup>	AVH, HBV	0.95	0.92
	Healed AVH, HBV	0.70	0.50
39. 66 <sup>1</sup>	AVH, HBV	1.03	0.68
	Healed AVH, HBV	1.71	1.39

[0346] As seen in Table 1, 19 of 32 sera from patients diagnosed as having NANBH were positive with respect to antibodies directed against HCV epitopes present in SOD-NANB<sub>5-1-1</sub> and SOD-NANB<sub>81</sub>.

[0347] However, the serum samples which were positive were not equally immunologically reactive with SOD-NANB<sub>5-1-1</sub> and SOD-NANB<sub>81</sub>. Serum samples from patient No. 1 were positive to SOD-NANB<sub>81</sub> but not to SOD-NANB<sub>5-1-1</sub>. Serum samples from patients number 10, 15, and 17 were positive to SOD-NANB<sub>5-1-1</sub> but not to SOD-NANB<sub>81</sub>. Serum samples from patients No. 3, 8, 11, and 12 reacted equally with both fusion polypeptides, whereas serum samples from patients No. 2, 4, 7, and 9 were 2-3 fold higher in the reaction to SOD-NANB<sub>5-1-1</sub> than to SOD-NANB<sub>81</sub>. These results suggest that NANB<sub>5-1-1</sub> and NANB<sub>81</sub> may contain at least 3 different epitopes; i.e., it is possible that each polypeptide contains at least 1 unique epitope, and that the two polypeptides share at least 1 epitope.

#### IV.D.4. Specificity of the Solid Phase RIA for NANBH

[0348] The specificity of the solid phase RIAs for NANBH was tested by using the assay on serum from patients infected with HAV or with HBV and on sera from control individuals. The assays utilizing partially purified SOD-NANB<sub>5-1-1</sub> and SOD-NANB<sub>81</sub> were conducted essentially as described in Section IV.D.3, except that the sera was from patients previously diagnosed as having HAV or HBV, or from individuals who were blood bank donors. The results for sera from HAV and HBV infected patients are presented in table 1. The RIA was tested using 11 serum specimens from HAV infected patients, and 20 serum specimens from HBV infected patients. As shown in table 1, none of these sera yielded a positive immunological reaction with the fusion polypeptides containing BB-NANBV epitopes.

[0349] The RIA using the NANB<sub>5-1-1</sub> antigen was used to determine immunological reactivity of serum from control individuals. Out of 230 serum samples obtained from the normal blood donor population, only 2 yielded positive reactions in the RIA (data not shown). It is possible that the two blood donors from whom these serum samples originated had previously been exposed to HCV.

#### IV.D.5. Reactivity of NANB<sub>5-1-1</sub> During the Course of NANBH Infection.

[0350] The presence of anti-NANB<sub>5-1-1</sub> antibodies during the course of NANBH infection of 2 patients and 4 chimpanzees was followed using RIA as described in Section IV.D.3. In addition the RIA was used to determine the presence or absence of anti-NANB<sub>5-1-1</sub> antibodies during the course of infection of HAV and HBV in infected chimpanzees.

[0351] The results, which are presented in Table 2, show that with chimpanzees and with humans, anti-NANB<sub>5-1-1</sub>

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antibodies were detected following the onset of the acute phase of NANBH infection. Anti-NANB<sub>5-1-1</sub> antibodies were not detected in serum samples from chimpanzees infected with either HAV or HBV. Thus anti-NANB<sub>5-1-1</sub> antibodies serve as a marker for an individual's exposure to HCV.

Table 2

Seroconversion in Sequential Serum Samples from Hepatitis Patients and Chimpanzees Using 5-1-1 Antigen				
Patient/ Chimp	Sample Date (Days) (o-Inoculation day)	Hepatitis Viruses	Anti-5-1-1 (S/N)	ALT (mu/ml)
Patient 29	T8	NANB	1.09	1180
	T+180		33.89	425
	T+208		36.22	--
Patient 30	T	NANB	1.90	1830
	T+307		34.17	290
	T+799		32.45	276
Chimp 1	0	NANB	0.87	9
	76		0.93	71
	118		23.67	19
	154		32.41	--
Chimp 2	0	NANB	1.00	5
	21		1.08	52
	73		4.64	13
	138		25.01	--
Chimp 3	0	NANB	1.08	8
	43		1.44	205
	53		1.82	14
	159		11.87	6
Chimp 4	-3	NANB	1.12	11
	55		1.25	132
	83		6.60	--
Chimp 5	140	HAV	17.51	--
	0		1.50	4
	25		2.39	147
Chimp 6	40	HAV	1.92	18
	268		1.53	5
	-8		0.85	--
	15		--	106
Chimp 7	41	HAV	0.81	10
	129		1.33	--
	0		1.17	7
	22		1.60	83
Chimp 8	115	HAV	1.55	5
	139		1.60	--
	0		0.77	15
	26		0.98	130
Chimp 9	74	HBV	1.77	8
	205		1.27	5
	-290		1.74	--
	379		3.29	9
Chimp 10	435	HBV	2.77	6
	0		2.35	8
	111-118 (pool)		2.74	96-156 (pool)
	205		2.05	9
	240		1.78	13

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Table 2 (continued)

Seroconversion In Sequential Serum Samples from Hepatitis Patients and Chimpanzees Using 5-1-1 Antigen				
Patient/ Chimp	Sample Date (Days) (o-Inoculation day)	Hepatitis Viruses	Anti-5-1-1 (S/N)	ALT (mu/ml)
Chimp 11	0	HVB	1.82	11
	28-56 (pool)		1.26	8-100 (pool)
	169		—	9
	223		0.52	10
*T=day of initial sampling				

IV.E. Purification of Polyclonal Serum Antibodies to NANB<sub>5-1-1</sub>

[0352] On the basis of the specific immunological reactivity of the SOD-NANB<sub>5-1-1</sub> polypeptide with the antibodies in serum samples from patients with NANBH, a method was developed to purify serum antibodies which react immunologically with the epitope(s) in NANB<sub>5-1-1</sub>. This method utilizes affinity chromatography. Purified SOD-NANB<sub>5-1-1</sub> polypeptide (see Section IV.D.1) was attached to an insoluble support; the attachment is such that the immobilized polypeptide retains its affinity for antibody to NANB<sub>5-1-1</sub>. Antibody in serum samples is absorbed to the matrix-bound polypeptide. After washing to remove non-specifically bound materials and unbound materials, the bound antibody is released from the bound SOD-HCV polypeptide by change in pH, and/or by chaotropic reagents, for example, urea.

[0353] Nitrocellulose membranes containing bound SOD-NANB<sub>5-1-1</sub> were prepared as follows. A nitrocellulose membrane, 2.1 cm Sartorius of 0.2 micron pore size, was washed for 3 minutes three times with BBS. SOD-NANB<sub>5-1-1</sub> was bound to the membrane by incubation of the purified preparation in BBS at room temperature for 2 hours; alternatively it was incubated at 4°C overnight. The solution containing unbound antigen was removed, and the filter was washed three times with BBS for three minutes per wash. The remaining active sites on the membrane were blocked with BSA by incubation with a 5 mg/ml BSA solution for 30 minutes. Excess BSA was removed by washing the membrane with 5 times with BBS and 3 times with distilled water. The membrane containing the viral antigen and BSA was then treated with 0.05 M glycine hydrochloride, pH 2.5, 0.10 M NaCl (GlyHCl) for 15 minutes, followed by 3 three minute washes with PBS.

[0354] Polyclonal anti-NANB<sub>5-1-1</sub> antibodies were isolated by incubating the membranes containing the fusion polypeptide with serum from an individual with NANBH for 2 hours. After the incubation, the filters were washed 5 times with BBS, and twice with distilled water. Bound antibodies were then eluted from each filter with 5 elutions of GlyHCl, at 3 minutes per elution. The pH of the eluates was adjusted to pH 8.0 by collecting each eluate in a test tube containing 2.0 M Tris HCl, pH 8.0. Recovery of the anti-NANB<sub>5-1-1</sub> antibody after affinity chromatography is approximately 50%.

[0355] The nitrocellulose membranes containing the bound viral antigen can be used several times without appreciable decrease in binding capacity. To reuse the membranes, after the antibodies have been eluted the membranes are washed with BBS three times for 3 minutes. They are then stored in BBS at 4°C.

## IV.F. The Capture of HCV Particles from Infected Plasma Using Purified Human Polyclonal Anti-HCV Antibodies; Hybridization of the Nucleic Acid in the Captured Particles to HCV cDNA

## IV.F.1. The Capture of HCV Particles from Infected Plasma Using Human Polyclonal Anti-HCV Antibodies

[0356] Protein-nucleic acid complexes present in infectious plasma of a chimpanzee with NANBH were isolated using purified human polyclonal anti-HCV antibodies which were bound to polystyrene beads.

[0357] Polyclonal anti-NANB<sub>5-1-1</sub> antibodies were purified from serum from a human with NANBH using the SOD-HCV polypeptide encoded in clone 5-1-1. The method for purification was that described in Section IV.E.

[0358] The purified anti-NANB<sub>5-1-1</sub> antibodies were bound to polystyrene beads (1/4" diameter, specular finish, Precision Plastic Ball Co., Chicago, Illinois) by incubating each at room temperature overnight with 1 ml of antibodies (1 microgram/ml in borate buffered saline, pH 8.5). Following the overnight incubation, the beads were washed once with TBST [50 mM Tris HCl, pH 8.0, 150 mM NaCl, 0.05% (v/v) Tween 20], and then with phosphate buffered saline (PBS) containing 10 mg/ml BSA.

[0359] Control beads were prepared in an identical fashion, except that the purified anti-NANB<sub>5-1-1</sub> antibodies were replaced with total human immunoglobulin.

[0360] Capture of HCV from NANBH infected chimpanzee plasma using the anti-NANB<sub>5-1-1</sub> antibodies bound to beads was accomplished as follows. The plasma from a chimpanzee with NANBH used is described in Section IV.A. 1.. An aliquot (1 ml) of the NANBV infected chimpanzee plasma was incubated for 3 hours at 37°C with each of 5 beads coated with either anti-NANB<sub>5-1-1</sub> antibodies, or with control immunoglobulins. The beads were washed 3 times

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with TBST.

#### IV.F.2. Hybridization of the Nucleic Acid in the Captured Particles to NANBV-cDNA

5 [0361] The nucleic acid component released from the particles captured with anti-NANB<sub>5-1-1</sub> antibodies was analyzed for hybridization to HCV cDNA derived from clone 81.

[0362] HCV particles were captured from NANBH infected chimpanzee plasma, as described in IV.F.1. To release the nucleic acids from the particles, the washed beads were incubated for 60 min. at 37°C with 0.2 ml per bead of a solution containing proteinase K (1 mg/ml), 10 mM Tris HCl, pH 7.5, 10 mM EDTA, 0.25% (w/v) SDS, 10 micrograms/ml soluble yeast RNA, and the supernatant solution was removed. The supernatant was extracted with phenol and chloroform, and the nucleic acids precipitated with ethanol overnight at -20 °C. The nucleic acid precipitate was collected by centrifugation, dried, and dissolved in 50 mM Hepes, pH 7.5. Duplicate aliquots of the soluble nucleic acids from the samples obtained from beads coated with anti-NANB<sub>5-1-1</sub> antibodies and with control beads containing total human immunoglobulin were filtered onto to nitrocellulose filters. The filters were hybridized with a <sup>32</sup>P-labeled, nick-translated probe made from the purified HCV cDNA fragment in clone 81. The methods for preparing the probe and for the hybridization are described in Section IV.C.1..

[0363] Autoradiographs of a probed filter containing the nucleic acids from particles captured by beads containing anti-NANB<sub>5-1-1</sub> antibodies are shown in Fig. 40. The extract obtained using the anti-NANB<sub>5-1-1</sub> antibody (A<sub>1</sub>, A<sub>2</sub>) gave clear hybridization signals relative to the control antibody extract (A<sub>3</sub>, A<sub>4</sub>) and to control yeast RNA (B<sub>1</sub>, B<sub>2</sub>). Standards consisting of 1pg, 5pg, and 10pg of the purified, clone 81 cDNA fragment are shown in C1-3, respectively.

20 [0364] These results demonstrate that the particles captured from NANBH plasma by anti-NANB<sub>5-1-1</sub> antibodies contain nucleic acids which hybridize with HCV cDNA in clone 81, and thus provide further evidence that the cDNAs in these clones are derived from the etiologic agent for NANBH.

#### 25 IV.G. Immunological Reactivity of C100-3 with Purified Anti-NANB<sub>5-1-1</sub> Antibodies

[0365] The immunological reactivity of C100-3 fusion polypeptide with anti-NANB<sub>5-1-1</sub> antibodies was determined by a radioimmunoassay, in which the antigens which were bound to a solid phase were challenged with purified anti-NANB<sub>5-1-1</sub> antibodies, and the antigen-antibody complex detected with <sup>125</sup>I-labeled sheep anti-human antibodies. The immunological reactivity of C100-3 polypeptide was compared with that of SOD-NANB<sub>5-1-1</sub> antigen.

30 [0366] The fusion polypeptide C100-3 was synthesized and purified as described in Section IV.B.5. and in Section IV.B.6., respectively. The fusion polypeptide SOD-NANB<sub>5-1-1</sub> was synthesized and purified as described in Section IV.B.1. and in Section IV.D.1., respectively. Purified anti-NANB<sub>5-1-1</sub> antibodies were obtained as described in Section IV.E.

35 [0367] One hundred microliter aliquots containing varying amounts of purified C100-3 antigen in 0.125M Na borate buffer, pH 8.3, 0.075M NaCl (BBS) was added to each well of a microtiter plate (Dynatech Immulon 2 Removawell Strips). The plate was incubated at 4°C overnight in a humid chamber, after which, the protein solution was removed and the wells washed 3 times with BBS containing 0.02% Triton X-100 (BBST). To prevent non-specific binding, the wells were coated with BSA by addition of 100 microliters of a 5 mg/ml solution of BSA in BBS followed by incubation at room temperature for 1 hour, after which the excess BSA solution was removed. The polypeptides in the coated wells were reacted with purified anti-NANB<sub>5-1-1</sub> antibodies by adding 1 microgram antibody/well, and incubating the samples for 1 hr at 37°C. After incubation, the excess solution was removed by aspiration, and the wells were washed 5 times with BBST. Anti-NANB<sub>5-1-1</sub> bound to the fusion polypeptides was determined by the binding of <sup>125</sup>I-labeled F' (ab)<sub>2</sub> sheep anti-human IgG to the coated wells. Aliquots of 100 microliters of the labeled probe (specific activity 5-20 microcuries/microgram) were added to each well, and the plates were incubated at 37°C for 1 hour, followed by removal of excess probe by aspiration, and 5 washes with BBST. The amount of radioactivity bound in each well was determined by counting in a counter which detects gamma radiation.

45 [0368] The results of the immunological reactivity of C100 with purified anti-NANB<sub>5-1-1</sub> as compared to that of NANB<sub>5-1-1</sub> with the purified antibodies are shown in Table 3.

Table 3

Immunological Reactivity of C100-3 compared to NANB <sub>5-1-1</sub> by Radioimmunoassay						
AG(ng)	RIA (cpm/assay)					
	400	320	240	160	60	0
NANB <sub>5-1-1</sub>	7332	6732	4954	4050	3051	57
C100-3	7450	6985	5920	5593	4096	67

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[0369] The results in Table 3 show that anti-NANB<sub>5-1-1</sub> recognizes an epitope(s) in the C100 moiety of the C100-3 polypeptide. Thus NANB<sub>5-1-1</sub> and C100 share a common epitope(s). The results suggest that the cDNA sequence encoding this NANBV epitope(s) is one which is present in both clone 5-1-1 and in clone 81.

#### 5 IV.H. Characterization of HCV

##### IV.H.1. Characterization of the Strandedness of the HCV Genome.

[0370] The HCV genome was characterized with respect to its strandedness by isolating the nucleic acid fraction from particles captured on anti-NANB<sub>5-1-1</sub> antibody coated polystyrene beads, and determining whether the isolated nucleic acid hybridized with plus and/or minus strands of HCV cDNA.

[0371] Particles were captured from HCV infected chimpanzee plasma using polystyrene beads coated with immunopurified anti-NANB<sub>5-1-1</sub> antibody as described in Section IV.F.1. The nucleic acid component of the particles was released using the method described in Section IV.F.2. Aliquots of the isolated genomic nucleic acid equivalent to 3 mls of high titer plasma were blotted onto nitrocellulose filters. As controls, aliquots of denatured HCV cDNA from clone 81 (2 picograms) was also blotted onto the same filters. The filters were probed with <sup>32</sup>P-labeled mixture of plus or mixture of minus strands of single stranded DNA cloned from HCV cDNAs; the cDNAs were excised from clones 40b, 81, and 25c.

[0372] The single stranded probes were obtained by excising the HCV cDNAs from clones 81, 40b, and 25c with EcoRI, and cloning the cDNA fragments in M13 vectors, mp18 and mp19 [Messing (1983)]. The M13 clones were sequenced to determine whether they contained the plus or minus strands of DNA derived from the HCV cDNAs. Sequencing was by the dideoxychain termination method of Sanger et al. (1977).

[0373] Each of a set of duplicate filters containing aliquots of the HCV genome isolated from the captured particles was hybridized with either plus or minus strand probes derived from the HCV cDNAs. Fig. 41 shows the autoradiographs obtained from probing the NANBV genome with the mixture of probes derived from clones 81, 40b, and 25c. This mixture was used to increase the sensitivity of the hybridization assay. The samples in panel I were hybridized with the plus strand probe mixture. The samples in panel II were probed by hybridization with the minus strand probe mixture. The composition of the samples in the panels of the immunoblot are presented in table 4.

Table 4

lane	A	B
1	HCV genome	*
2	----	*
3	*	cDNA 81
4	----	cDNA 81

\* is an undescribed sample.

[0374] As seen from the results in Fig. 41, only the minus strand DNA probe hybridizes with the isolated HCV genome. This result, in combination with the result showing that the genome is sensitive to RNase and not DNase (See Section IV.C.2.), suggests that the genome of NANBV is positive stranded RNA.

[0375] These data, and data from other laboratories concerning the physicochemical properties of a putative NANBV (s), are consistent with the possibility that HCV is a member of the Flaviviridae. However, the possibility that HCV represents a new class of viral agent has not been eliminated.

##### IV.H.2. Detection of Sequences In Captured Particles Which When Amplified by PCR Hybridize to HCV cDNA Derived from Clone 81

[0376] The RNA in captured particles was obtained as described in Section IV.H.1. The analysis for sequences which hybridize to the HCV cDNA derived from clone 81 was carried out utilizing the PCR amplification procedure, as described in Section IV.C.3, except that the hybridization probe was a kinased oligonucleotide derived from the clone 81 cDNA sequence. The results showed that the amplified sequences hybridized with the clone 81 derived HCV cDNA probe.

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IV.H.3. Homology Between the Non-Structural Protein of Dengue Flavivirus (MNWVD1) and the HCV Polypeptides Encoded by the Combined ORF of Clones 14I Through 39c

[0377] The combined HCV cDNAs of clones 14I through 39c contain one continuous ORF, as shown in Fig. 26. The polypeptide encoded therein was analyzed for sequence homology with the region of the non-structural polypeptide (s) in Dengue flavivirus (MNWVD1). The analysis used the Dayhoff protein data base, and was performed on a computer. The results are shown in Fig. 42, where the symbol (:) indicates an exact homology, and the symbol (.) indicates a conservative replacement in the sequence; the dashes indicate spaces inserted into the sequence to achieve the greatest homologies. As seen from the figure, there is significant homology between the sequence encoded in the HCV cDNA, and the non-structural polypeptide(s) of Dengue virus. In addition to the homology shown in Fig. 42, analysis of the polypeptide segment encoded in a region towards the 3'-end of the cDNA also contained sequences which are homologous to sequences in the Dengue polymerase. Of consequence is the finding that the canonical Gly-Asp-Asp (GDD) sequence thought to be essential for RNA-dependent RNA polymerases is contained in the polypeptide encoded in HCV cDNA, in a location which is consistent with that in Dengue 2 virus. (Data not shown.)

IV.H.4. HCV-DNA Is Not Detectable in NANBH Infected Tissue

[0378] Two types of studies provide results suggesting that HCV-DNA is not detectable in tissue from an individual with NANBH. These results, in conjunction with those described in IV.C. and IV.H.1. and IV.H.2. provide evidence that HCV is not a DNA containing virus, and that its replication does not involve cDNA.

IV.H.4.a. Southern Blotting Procedure

[0379] In order to determine whether NANBH infected chimpanzee liver contains detectable HCV-DNA (or HCV-cDNA), restriction enzyme fragments of DNA isolated from this source was Southern blotted, and the blots probed with <sup>32</sup>P-labeled HCV cDNA. The results showed that the labeled HCV cDNA did not hybridize to the blotted DNA from the infected chimpanzee liver. It also did not hybridize to control blotted DNA from normal chimpanzee liver. In contrast, in a positive control, a labeled probe of the beta-interferon gene hybridized strongly to Southern blots of restriction enzyme digested human placental DNA. These systems were designed to detect a single copy of the gene which was to be detected with the labeled probe.

[0380] DNAs were isolated from the livers of two chimpanzees with NANBH. Control DNAs were isolated from uninfected chimpanzee liver, and from human placentas. The procedure for extracting DNA was essentially according to Maniatis et al. (1982), and the DNA samples were treated with RNase during the isolation procedure.

[0381] Each DNA sample was treated with either EcoRI, MboI, or HincII (12 micrograms), according to the manufacturer's directions. The digested DNAs were electrophoresed on 1% neutral agarose gels, Southern blotted onto nitrocellulose, and the blotted material hybridized with the appropriate nick-translated probe cDNA (3 x 10<sup>6</sup> cpm/ml of hybridization mix). The DNA from infected chimpanzee liver and normal liver were hybridized with <sup>32</sup>P-labeled HCV cDNA from clones 36 plus 81; the DNA from human placenta was hybridized with <sup>32</sup>P-labeled DNA from the beta-interferon gene. After hybridization, the blots were washed under stringent conditions, i.e., with a solution containing 0.1 x SSC, 0.1% SDS, at 65°C.

[0382] The beta-interferon gene DNA was prepared as described by Houghton et al (1981).

IV.H.4.b. Amplification by the PCR Technique

[0383] In order to determine whether HCV-DNA could be detected in liver from chimpanzees with NANBH, DNA was isolated from the tissue, and subjected to the PCR amplification-detection technique using primers and probe polynucleotides derived from HCV cDNA from clone 81. Negative controls were DNA samples isolated from uninfected HepG2 tissue culture cells, and from presumably uninfected human placenta. Positive controls were samples of the negative control DNAs to which a known relatively small amount (250 molecules) of the HCV cDNA insert from clone 81 was added.

[0384] In addition, to confirm that RNA fractions isolated from the same livers of chimpanzees with NANBH contained sequences complementary to the HCV-cDNA probe, the PCR amplification-detection system was also used on the isolated RNA samples.

[0385] In the studies, the DNAs were isolated by the procedure described in Section IV.H.4.a, and RNAs were extracted essentially as described by Chirgwin et al. (1981).

[0386] Samples of DNA were isolated from 2 infected chimpanzee livers, from uninfected HepG2 cells, and from human placenta. One microgram of each DNA was digested with HindIII according to the manufacturer's directions. The digested samples were subjected to PCR amplification and detection for amplified HCV cDNA essentially as de-

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scribed in Section IV.C.3., except that the reverse transcriptase step was omitted. The PCR primers and probe were from HCV cDNA clone 81, and are described in Section IV.C.3.. Prior to the amplification, for positive controls, a one microgram sample of each DNA was "spiked" by the addition of 250 molecules of HCV cDNA Insert Isolated from clone 81.

**[0387]** In order to determine whether HCV sequences were present in RNA isolated from the livers of chimpanzees with NANBH, samples containing 0.4 micrograms of total RNA were subjected to the amplification procedure essentially as described in Section IV.C.3., except that the reverse transcriptase was omitted from some of the samples as a negative control. The PCR primers and probe were from HCV cDNA clone 81, as described supra.

**[0388]** The results showed that amplified sequences complementary to the HCV cDNA probe were not detectable in the DNAs from infected chimpanzee liver, nor were they detectable in the negative controls. In contrast, when the samples, including the DNA from infected chimpanzee liver, was spiked with the HCV cDNA prior to amplification, the clone 81 sequences were detected in all positive control samples. In addition, in the RNA studies, amplified HCV cDNA clone 81 sequences were detected only when reverse transcriptase was used, suggesting strongly that the results were not due to a DNA contamination.

**[0389]** These results show that hepatocytes from chimpanzees with NANBH contain no, or undetectable levels, of HCV DNA. Based upon the spiking study, if HCV DNA is present, it is at a level far below .06 copies per hepatocyte. In contrast, the HCV sequences in total RNA from the same liver samples was readily detected with the PCR technique.

#### IV.I. ELISA Determinations for HCV Infection Using HCV c100-3 As Test Antigen

**[0390]** All samples were assayed using the HCV c100-3 ELISA. This assay utilizes the HCV c100-3 antigen (which was synthesized and purified as described in Section IV.B.5), and a horseradish peroxidase (HRP) conjugate of mouse monoclonal anti-human IgG.

**[0391]** Plates coated with the HCV c100-3 antigen were prepared as follows. A solution containing Coating buffer (50mM Na Borate, pH 9.0), 21 ml/plate, BSA (25 micrograms/ml), c100-3 (2.50 micrograms/ml) was prepared just prior to addition to the Removeawell Immulon I plates (Dynatech Corp.). After mixing for 5 minutes, 0.2ml/well of the solution was added to the plates, they were covered and incubated for 2 hours at 37°C, after which the solution was removed by aspiration. The wells were washed once with 400 microliters Wash Buffer (100 mM sodium phosphate, pH 7.4, 140 mM sodium chloride, 0.1% (W/V) casein, 1% (W/V) Triton x-100, 0.01% (W/V) Thimerosal). After removal of the wash solution, 200 microliters/well of Postcoat solution (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 0.1% (w/v) casein and 2 mM phenylmethylsulfonylfluoride (PMSF)) was added, the plates were loosely covered to prevent evaporation, and were allowed to stand at room temperature for 30 minutes. The wells were then aspirated to remove the solution, and lyophilized dry overnight, without shelf heating. The prepared plates may be stored at 2-8°C in sealed aluminum pouches.

**[0392]** In order to perform the ELISA determination, 20 microliters of serum sample or control sample was added to a well containing 200 microliters of sample diluent (100 mM sodium phosphate, pH 7.4, 500 mM sodium chloride, 1 mM EDTA, 0.1% (W/V) Casein, 0.015 (W/V) Therosal, 1% (W/V) Triton X-100, 100 micrograms/ml yeast extract). The plates were sealed, and incubated at 37°C for two hours, after which the solution was removed by aspiration, and the wells were washed with 400 microliters of wash buffer (phosphate buffered saline (PBS) containing 0.05% Tween 20). The washed wells were treated with 200 microliters of mouse anti-human IgG-HRP conjugate contained in a solution of Ortho conjugate diluent (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 50% (V/V) fetal bovine serum, 1% (V/V) heat treated horse serum, 1 mM K<sub>3</sub>Fe (CN)<sub>6</sub>, 0.05% (W/V) Tween 20, 0.02% (W/V) Thimerosal). Treatment was for 1 hour at 37°C, the solution was removed by aspiration, and the wells were washed with wash buffer, which was also removed by aspiration. To determine the amount of bound enzyme conjugate, 200 microliters of substrate solution (10 mg O-phenylenediamine dihydrochloride per 5 ml of Developer solution) was added. Developer solution contains 50 mM sodium citrate adjusted to pH 5.1 with phosphoric acid, and 0.6 microliters/ml of 30% H<sub>2</sub>O<sub>2</sub>. The plates containing the substrate solution were incubated in the dark for 30 minutes at room temperature, the reactions were stopped by the addition of 50 microliters/ml 4N sulfuric acid, and the ODs determined.

**[0393]** The examples provided below show that the microtiter plate screening ELISA which utilizes HCV c100-3 antigen has a high degree of specificity, as evidenced by an initial rate of reactivity of about 1%, with a repeat reactive rate of about 0.5% on random donors. The assay is capable of detecting an immunoreponse in both the post acute phase of the infection, and during the chronic phase of the disease. In addition, the assay is capable of detecting some samples which score negative in the surrogate tests for NANBH; these samples come from individuals with a history of NANBH, or from donors implicated in NANBH transmission.

**[0394]** In the examples described below, the following abbreviations are used:

ALT	Alanine amino transferase
Anti-HBc	Antibody against HBc

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	Anti-HBsAg	Antibody against HBsAg
	HBc	Hepatitis B core antigen
	ABsAg	Hepatitis B surface antigen
	IgG	Immunoglobulin G
5	IgM	Immunoglobulin M
	IU/L	International units/Liter
	NA	Not available
	NT	Not tested
	N	Sample size
10	Neg	Negative
	OD	Optical density
	Pos	Positive
	S/CO	Signal/cutoff
	SD	Standard deviation
15	x	Average or mean
	WNL	Within normal limits

IV.1.1. HCV Infection in a Population of Random Blood Donors

20 [0395] A group of 1,056 samples (fresh sera) from random blood donors were obtained from Irwin Memorial Blood Bank, San Francisco, California. The test results obtained with these samples are summarized in a histogram showing the distribution of the OD values (Fig. 43). As seen in Fig. 43, 4 samples read >3, 1 sample reads between 1 and 3, 5 samples read between 0.4 and 1, and the remaining 1,046 samples read <0.4, with over 90% of these samples reading <0.1.

25 [0396] The results on the reactive random samples are presented in Table 5. Using a cut-off value equal to the mean plus 5 standard deviations, ten samples out of the 1,056 (0.95%) were initially reactive. Of these, five samples (0.47%) repeated as reactive when they were assayed a second time using the ELISA. Table 5 also shows the ALT and Anti-HBc status for each of the repeatedly reactive samples. Of particular interest is the fact that all five repeat reactive samples were negative in both surrogate tests for NANBH, while scoring positive in the HCV ELISA.

TABLE 5

RESULTS ON REACTIVE RANDOM SAMPLES				
N = 1051				
$\bar{x} = 0.049^*$				
SD = $\pm 0.074$				
Cut-off: $\bar{x} + 5SD = 0.419$ (0.400 + Negative Control)				
Samples	Initial Reactives OD	Repeat Reactives OD	ALT**	Anti HBc***
			(IU/L)	(OD)
4227	0.462	0.084	NA	NA
6292	0.569	0.294	NA	NA
6188	0.699	0.326	NA	NA
6157	0.735	0.187	NA	NA
6277	0.883	0.152	NA	NA
6397	1.567	1.392	30.14	1.433
6019	>3.000	>3.000	46.48	1.057
6651	>3.000	>3.000	48.53	1.343
6669	>3.000	>3.000	60.53	1.165
4003	>3.000	3.000	WNL****	Negative
	10/1056 = 0.95%	5/1056 = 0.47%		

\* Samples reading >1.5 were not included in calculating the Mean and SD

\*\* ALT  $\geq 68$  IU/L is above normal limits.

\*\*\* Anti-HBc  $\leq 0.535$  (competition assay) is considered positive.

\*\*\*\* WNL: Within normal limits.

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## IV.1.2. Chimpanzee Serum Samples

[0397] Serum samples from eleven chimpanzees were tested with the HCV c100-3 ELISA. Four of these chimpanzees were infected with NANBH from a contaminated batch of Factor VIII (presumably Hutchinson strain), following an established procedure in a collaboration with Dr. Daniel Bradley at the Centers for Disease Control. As controls, four other chimpanzees were infected with HAV and three with HBV. Serum samples were obtained at different times after infection.

[0398] The results, which are summarized in Table 6, show documented antibody seroconversion in all chimpanzees infected with the Hutchinson strain of NANBH. Following the acute phase of infection (as evidenced by the significant rise and subsequent return to normal of ALT levels), antibodies to HCV c100-3 became detectable in the sera of the 4/4 NANBH infected chimpanzees. These samples had previously been shown, as discussed in Section IV.B.3., to be positive by a Western analysis, and an RIA. In contrast, none of the control chimpanzees which had been infected with HAV or HBV showed evidence of reactivity in the ELISA.

TABLE 6

CHIMPANZEE SERUM SAMPLES						
	OD	S/CO	INOCULATION DATE	BLEED DATE	ALT (IU/L)	TRANSFUSED
NEGATIVE CONTROL	0.001					
POSITIVE CONTROL	1.504					
CUTOFF	0.401					
<u>Chimp 1</u>	-0.007	0.00	05/24/81	05/21/84	9	NANB
	0.003	0.01		08/07/81	71	
	>3.000	>7.48		09/18/84	19	
	>3.000	>7.48		10/24/84	---	
<u>Chimp 2</u>	---	---	06/07/84	---	---	NANB
	-0.003	0.00		05/31/84	5	
	-0.005	0.00		06/28/84	52	
	0.945	2.36		08/20/84	13	
	>3.000	>7.48		10/24/84	---	
<u>Chimp 3</u>	0.005	0.01	03/14/85	03/14/85	8	NANB
	0.017	0.04		04/26/85	205	
	0.006	0.01		05/06/85	14	
	1.010	2.52		08/20/85	6	
<u>Chimp 4</u>	-0.006	0.00	03/11/85	03/11/85	11	NANB
	0.003	0.01		05/09/85	132	
	0.523	1.31		06/06/85	---	
	1.574	3.93		08/01/85	---	
<u>Chimp 5</u>	-0.006	0.00	11/21/80	11/21/80	4	HAV
	0.001	0.00		12/16/80	147	
	0.003	0.01		12/30/80	18	
	0.006	0.01		07/29 - 08/21/81	5	
<u>Chimp 6</u>	---	---	05/25/82	---	---	HAV
	-0.005	0.00		05/17/82	---	
	0.001	0.00		06/10/82	106	
	-0.004	0.00		07/06/82	10	
	0.290	0.72		10/01/82	---	
<u>Chimp 7</u>	-0.008	0.00	05/25/82	05/25/82	7	HAV
	-0.004	0.00		06/17/82	83	
	-0.006	0.00		09/16/82	5	
	0.005	0.01		10/09/82	---	

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TABLE 6 (continued)

CHIMPANZEE SERUM SAMPLES							
	OD	S/CO	INOCULATION DATE	BLEED DATE	ALT (IU/L)	TRANSFUSED	
5	<u>Chimp 8</u>	-0.007	0.00	11/21/80	11/21/80	15	INV
		0.000	0.00		12/16/80	130	
		0.004	0.01		02/03/81	8	
		0.000	0.00		06/03 - 06/10/81	4.5	
10	<u>Chimp 9</u>	---	---	07/24/80	---	---	HBV
		0.019	0.05		08/22 - 10/10/79	---	
		---	---		03/11/81	57	
		0.015	0.01		07/01 - 08/05/81	9	
		0.008	0.02		10/01/81	6	
15	<u>Chimp 10</u>	---	---	05/12/82	---	---	HBV
		0.011	0.03		04/21 - 05/12/82	9	
		0.015	0.04		09/01 - 09/08/82	126	
		0.008	0.02		12/02/82	9	
20		0.010	0.02		01/06/83	13	
	<u>Chimp 11</u>	---	---	05/12/82	---	---	HBV
		0.000	0.00		01/06 - 05/12/82	11	
		---	---		06/23/82	100	
25		-0.003	0.00		06/09 - 07/07/82	---	
		-0.003	0.00		10/28/82	9	
		-0.003	0.00		12/20/82	10	

## IV.I.3. Panel 1: Proven Infectious Sera from Chronic Human NANBH Carriers

[0399] A coded panel consisted of 22 unique samples, each one in duplicate, for a total of 44 samples. The samples were from proven infectious sera from chronic NANBH carriers, infectious sera from implicated donors, and infectious sera from acute phase NANBH patients. In addition, the samples were from highly pedigreed negative controls, and other disease controls. This panel was provided by Dr. H. Alter of the Department of Health and Human Services, National Institutes of Health, Bethesda, Maryland. The panel was constructed by Dr. Alter several years ago, and has been used by Dr. Alter as a qualifying panel for putative NANBH assays.

[0400] The entire panel was assayed twice with the ELISA assay, and the results were sent to Dr. Alter to be scored. The results of the scoring are shown in Table 7. Although the Table reports the results of only one set of duplicates, the same values were obtained for each of the duplicate samples.

[0401] As shown in Table 7, 6 sera which were proven infectious in a chimpanzee model were strongly positive. The seventh infectious serum corresponded to a sample for an acute NANBH case, and was not reactive in this ELISA. A sample from an implicated donor with both normal ALT levels and equivocal results in the chimpanzee studies was non-reactive in the assay. Three other serial samples from one individual with acute NANBH were also non-reactive. All samples coming from the highly pedigreed negative controls, obtained from donors who had at least 10 blood donations without hepatitis implication, were non-reactive in the ELISA. Finally, four of the samples tested had previously scored as positive in putative NANBH assays developed by others, but these assays were not confirmable. These four samples scored negatively with the HCV ELISA.

TABLE 7

50	H. ALTER'S PANEL 1:						
	PANEL				1ST RESULT	2ND RESULT	
	1)	PROVEN INFECTIOUS BY CHIMPANZEE TRANSMISSION					
		A.	CUROUIC NANB; POST-TX				
55			JF		+	+	
			EB		+	+	

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TABLE 7 (continued)

H. ALTER'S PANEL 1:				
PANEL			1ST RESULT	2ND RESULT
5		PG	+	+
	B.	IMPLICATED DONORS WITH ELEVATED ALT		
		BC	+	+
		JJ	+	+
		BB	+	+
10	C.	ACUTE NANB; POST-TX		
		WII	-	-
	2)	EQUIVOCALLY INFECTIOUS BY CHIMPANZEE TRANSMISSION		
	A.	IMPLICATED DONOR WITH NORMAL ALT		
15		CC	-	-
		JL WEEK 1	-	-
		JL WEEK 2	-	-
		JL WEEK 3	-	-
	4)	DISEASE CONTROLS		
20	A.	PRIMARY BILIARY CIRRHOSIS		
		EK	-	-
	B.	ALCOHOLIC HEPATITIS IN RECOVERY		
		HB	-	-
25	5)	PEDIGREED NEGATIVE CONTROLS		
		DH	-	-
		DC	-	-
		LV	-	-
		ML	-	-
30		All	-	-
	6)	POTENTIAL NANB "ANTIGENS"		
		JS-80-01T-0 (ISHIDA)	-	-
		ASTERIX ( TREPO)	-	-
		ZURTZ (ARNOLD)	-	-
35		BECASSDINE (TREPO)	-	-

## IV.I.4. Panel 2: Donor/Recipient NANBH

40 [0402] The coded panel consisted of 10 unequivocal donor-recipient cases of transfusion associated NANBH, with a total of 188 samples. Each case consisted of samples of some or all the donors to the recipient, and of serial samples (drawn 3, 6, and 12 months after transfusion) from the recipient. Also included was a pre-bleed, drawn from the recipient before transfusion. The coded panel was provided by Dr. H. Alter, from the NIH, and the results were sent to him for scoring.

45 [0403] The results, which are summarized in Table 8, show that the ELISA detected antibody seroconversion in 9 of 10 cases of transfusion associated NANBH. Samples from case 4 (where no seroconversion was detected), consistently reacted poorly in the ELISA. Two of the 10 recipient samples were reactive at 3 months post transfusion. At six months, 8 recipient samples were reactive; and at twelve months, with the exception of case 4, all samples were reactive. In addition, at least one antibody positive donor was found in 7 out of the 10 cases, with case 10 having two positive donors. Also, in case 10, the recipient's pre-bleed was positive for HCV antibodies. The one month bleed from this recipient dropped to borderline reactive levels, while it was elevated to positive at 4 and 10 month bleeds. Generally, a S/CO of 0.4 is considered positive. Thus, this case may represent a prior infection of the individual with HCV.

50 [0404] The ALT and HBc status for all the reactive, i.e., positive, samples are summarized in Table 9. As seen in the table, 1/8 donor samples was negative for the surrogate markers and reactive in the HCV antibody ELISA. On the other hand, the recipient samples (followed up to 12 months after transfusion) had either elevated ALT, positive Anti-HBc, or both.

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TABLE 8

DONOR/RECIPIENT NANB PANEL										
H. ALTER DONOR/RECIPIENT NANB PANEL										
CASE	DONOR		RECIPIENT PRESLEED		3 MONTHS		POST-TX 6 MONTHS		12 MONTHS	
	OD	S/CD	OD	S/CD	OD	S/CD	OD	S/CD	OD	S/CO
1.	---	---	.032	0.07	.112	0.26	>3.000	>6.96	>3.000	>6.96
2.	---	---	.059	0.14	.050	0.12	1.681	3.90	>3.000	>6.96
3.	.403	0.94	.049	0.11	.057	0.13	>3.000	>6.96	>3.000	>6.96
4.	---	---	.065	0.15	.073	0.17	.067	0.16	.217	0.50
5.	>3.000	>6.96	.034	0.08	.096	0.22	>3.000	>6.96	>3.000	>6.96
6.	>3.000	>6.96	.056	0.13	1.475	3.44	>3.000	>6.96	>3.000	>6.96
7.	>3.000	>6.96	.034	0.08	.056	0.13	>3.000	>6.96	>3.000	>6.96
8.	>3.000	>6.96	.061	0.14	.078	0.18	2.262	5.28	>3.000	>6.96
9.	>3.000	>6.96	.080	0.19	.127	0.30	.055	0.13	>3.000	>6.96
10.	>3.000	>6.96	>3.000	>6.96	.317*	0.74	>3.000**	>6.96	>3.000***	>6.96
	>3.000	>6.96								

\* 1 MONTH,

\*\* 4 MONTHS,

\*\*\* 10 MONTHS

TABLE 9

ALT AND HBc STATUS FOR REACTIVE SAMPLES IN H. ALTER PANEL 1			
Samples		Anti-ALT*	HBc**
Donors			
Case 3		Normal	Negative
Case 5		Elevated	Positive
Case 6		Elevated	Positive
Case 7		Not available	Negative
Case 8		Normal	Positive
Case 9		Elevated	Not available
Case 10		Normal	Positive
Case 10		Normal	Positive
Recipients			
Case 1	6 mo	Elevated	Positive
12 mo	Elevated	Not tested	
Case 2	6 mo	Elevated	Negative
12 mo	Elevated	Not tested	
Case 3	6 mo	Normal	Not tested***
12 mo	Elevated	Not tested***	
Case 5	6 mo	Elevated	Not tested
12 mo	Elevated	Not tested	
Case 6	3 mo	Elevated	Negative

\* ALT  $\geq 45$  IU/L is above normal limits.\*\* Anti-HBc  $\leq 50\%$  (competition assay) is considered positive.

\*\*\* Prebleed and 3 mo samples were negative for HBc.

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TABLE 9 (continued)

ALT AND HBc STATUS FOR REACTIVE SAMPLES IN H. ALTER PANEL 1			
Samples		Anti-ALT*	HBc**
Recipients			
6 mo	Elevated	Negative	Negative
12 mo	Elevated	Not tested	
Case 7	6 mo	Elevated	Positive
12 mo	Elevated	Negative	
Case 8	6 mo	Normal	Not tested
12 mo	Elevated	Not tested	
Case 9	12 mo	Elevated	Not tested
Case 10	4 mo	Elevated	
10 mo	Elevated	Not tested	

\* ALT  $\geq 45$  IU/L is above normal limits.\*\* Anti-HBc  $\leq 50\%$  (competition assay) is considered positive.

## IV.1.5. Determination of HCV Infection in High Risk Group Samples

[0405] Samples from high risk groups were monitored using the ELISA to determine reactivity to HCV c100-3 antigen. These samples were obtained from Dr. Gary Tegtmeler, Community Blood Bank, Kansas City. The results are summarized in Table 10.

[0406] As shown in the table, the samples with the highest reactivity are obtained from hemophiliacs (76%). In addition, samples from individuals with elevated ALT and positive for Anti-HBc, scored 51% reactive, a value which is consistent with the value expected from clinical data and NANBH prevalence in this group. The incidence of antibody to HCV was also higher in blood donors with elevated ALT alone, blood donors positive for antibodies to Hepatitis B core alone, and in blood donors rejected for reasons other than high ALT or anti-core antibody when compared to random volunteer donors.

TABLE 10

NANBH HIGH RISK GROUP SAMPLES					
Group		N	Distribution		% Reactive
			N	OD	
Elevated ALT		35	3	>3.000	11.4%
	1	0.728			
Anti-HBc		24	5	>3.000	20.8%
Elevated ALT, Anti-HBc		33	12	>3.000	51.5%
	1	2.768			
	1	2.324			
	1	0.939			
	1	0.951			
	1	0.906			
Rejected Donors		25	5	>3.000	20.0%
Donors with History of Hepatitis		150	19	>3.000	14.7%
	1	0.837			
	1	0.714			
	1	0.469			

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TABLE 10 (continued)

NANBH HIGH RISK GROUP SAMPLES					
Group		N	Distribution		% Reactive
			N	OD	
Haemophiliacs		50	31	>3.000	76.0%
	1	2.568			
	1	2.483			
	1	2.000			
	1	1.979			
	1	1.495			
	1	1.209			
	1	0.819			

IV.1.6 Comparative Studies Using Anti-IgG or Anti-IgM Monoclonal Antibodies, or Polyclonal Antibodies as a Second Antibody in the HCV c100-3 ELISA

[0407] The sensitivity of the ELISA determination which uses the anti-IgG monoclonal conjugate was compared to that obtained by using either an anti-IgM monoclonal conjugate, or by replacing both with a polyclonal antiserum reported to be both heavy and light chain specific. The following studies were performed.

IV.1.6.a. Serial Samples from Seroconverters

[0408] Serial samples from three cases of NANB seroconverters were studied in the HCV c100-3 ELISA assay using in the enzyme conjugate either the anti-IgG monoclonal alone, or in combination with an anti-IgM monoclonal, or using a polyclonal antiserum. The samples were provided by Dr. Cladd Stevens, N.Y. Blood Center, N.Y.C., N.Y.. The sample histories are shown in Table 11.

[0409] The results obtained using an anti-IgG monoclonal antibody-enzyme conjugate are shown in Table 12. The data shows that strong reactivity is initially detected in samples 1-4, 2-8, and 3-5, of cases 1, 2, and 3, respectively.

[0410] The results obtained using a combination of an anti-IgG monoclonal conjugate and an anti-IgM conjugate are shown in Table 13. Three different ratios of anti-IgG to anti-IgM were tested; the 1:10,000 dilution of anti-IgG was constant throughout. Dilutions tested for the anti-IgM monoclonal conjugate were 1:30,000, 1:60,000, and 1:120,000. The data shows that, in agreement with the studies with anti-IgG alone, initial strong reactivity is detected in samples 1-4, 2-8, and 3-5.

[0411] The results obtained with the ELISA using anti-IgG monoclonal conjugate (1:10,000 dilution), or Tago polyclonal conjugate (1:80,000 dilution), or Jackson polyclonal conjugate (1:80,000 dilution) are shown in Table 14. The data indicates that initial strong reactivity is detected in samples 1-4, 2-8, and 3-5 using all three configurations; the Tago polyclonal antibodies yielded the lowest signals.

[0412] The results presented above show that all three configurations detect reactive samples at the same time after the acute phase of the disease (as evidenced by the ALT elevation). Moreover, the results indicate that the sensitivity of the HCV c100-3 ELISA using anti-IgG monoclonal-enzyme conjugate is equal to or better than that obtained using the other tested configurations for the enzyme conjugate.

TABLE 11

DESCRIPTION OF SAMPLES FROM CLADD STEVENS PANEL						
	Date	HBsAg	Anti-HBs	Anti-HBc	ALT	Bilirubin
Case 1						
1-1	8/5/81	1.0	91.7	12.9	40.0	-1.0
1-2	9/2/81	1.0	121.0	15.1	274.0	1.4
1-3	10/7/81	1.0	64.0	23.8	261.0	0.9
1-4	11/19/81	1.0	67.3	33.8	75.0	0.9

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TABLE 11 (continued)

DESCRIPTION OF SAMPLES FROM CLADD STEVENS PANEL						
	Date	HBsAg	Anti-HBs	Anti-HBc	ALT	Bilirubin
Case 1						
1-5	12/15/81	1.0	50.5	27.6	71.0	1.0
Case 2						
2-1	10/19/81	1.0	1.0	116.2	17.0	-1.0
2-2	11/17/81	1.0	0.8	89.5	46.0	1.1
2-3	12/02/81	1.0	1.2	78.3	63.0	1.4
2-4	12/14/81	1.0	0.9	90.6	152.0	1.4
2-5	12/23/81	1.0	0.8	93.6	624.0	1.7
2-6	1/20/82	1.0	0.8	92.9	66.0	1.5
2-7	2/15/82	1.0	0.8	86.7	70.0	1.3
2-8	3/17/82	1.0	0.9	69.8	24.0	-1.0
2-9	4/21/82	1.0	0.9	67.1	53.0	1.5
2-10	3/19/82	1.0	0.5	74.8	95.0	1.6
2-11	6/14/82	1.0	0.8	82.9	37.0	-1.0
Case 3						
3-1	4/7/81	1.0	1.2	88.4	13.0	-1.0
3-2	5/12/81	1.0	1.1	126.2	236.0	0.4
3-3	5/30/81	1.0	0.7	99.9	471.0	0.2
3-4	6/9/81	1.0	1.2	110.8	315.0	0.4
3-5	7/6/81	1.0	1.1	89.9	273.0	0.4
3-6	8/10/81	1.0	1.0	118.2	158.0	0.4
3-7	9/8/81	1.0	1.0	112.3	84.0	0.3
3-8	10/14/81	1.0	0.9	102.5	180.0	0.5
3-9	11/11/81	1.0	1.0	84.6	154.0	0.3

TABLE 12

ELISA RESULTS OBTAINED USING AN ANTI-IgG MONOCLONAL CONJUGATE				
SAMPLE	DATE	ALT	OD	S/CO
NEG CONTROL			.076	
CUTOFF			.476	
PC (1:128)			1.390	
CASE #1				
1-1	08/05/81	40.0	.178	.37
1-2	09/02/81	274.0	.154	.32
1-3	10/07/81	261.0	.129	.27
1-4	11/19/81	75.0	.937	1.97
1-5	12/15/81	71.0	>3.000	>6.30
CASE #2				
2-1	10/19/81	17.0	.058	0.12
2-2	11/17/81	46.0	.050	0.11
2-3	12/02/81	63.0	.047	0.10
2-4	12/14/81	152.0	.059	0.12
2-5	12/23/81	624.0	.070	0.15
2-6	01/20/82	66.0	.051	0.11

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TABLE 12 (continued)

ELISA RESULTS OBTAINED USING AN ANTI-IgG MONOCLONAL CONJUGATE				
SAMPLE	DATE	ALT	OD	S/CO
CASE #2				
2-7	02/15/82	70.0	.139	0.29
2-8	03/17/82	24.0	1.867	3.92
2-9	04/21/82	53.0	>3.000	>6.30
2-10	05/19/82	95.0	>3.000	>6.30
2-11	06/14/82	37.0	>3.000	>6.30
CASE #3				
3-1	04/07/81	13.0	.090	.19
3-2	05/12/81	236.0	.064	.13
3-3	05/30/81	471.0	.079	.17
3-4	06/09/81	315.0	.211	.44
3-5	07/06/81	273.0	1.707	3.59
3-6	08/10/81	158.0	>3.000	>6.30
3-7	09/08/81	84.0	>3.000	>6.30
3-8	10/14/81	180.0	>3.000	>6.30
3-9	11/11/81	154.0	>3.000	>6.30

TABLE 13

ELISA RESULTS OBTAINED USING ANTI-IgG and ANTI-IgM MONOCLONAL CONJUGATE							
			NANB ELISAs				
			MONOCLONALS		MONOCLONALS		MONOCLONALS
			IgG 1:10K IgM 1:30K		IgG 1:10K IgM 1:60K		IgG 1:10K IgM 1:120K
SAMPLE	DATE	ALT	OD	S/CO	OD	S/CO	OD S/CO
NEG CONTROL			.100		.080		.079
CUTOFF							
PC (1:128)			1.083		1.328		1.197
CASE #1							
1-1	08/05/81	40	.173		.162		.070
1-2	09/02/81	274	.194		.141		.079
1-3	10/07/81	261	.162		.129		.063
1-4	11/19/81	75	.312		.85		.709
1-5	12/15/81	71	>3.00		>3.00		>3.00
CASE #2							
2-1	10/19/81	17	.442		.045		.085
2-2	11/17/81	46	.102		.029		.030
2-3	12/02/81	63	.059		.036		.027
2-4	12/14/81	152	.065		.041		.025
2-5	12/23/81	624	.082		.033		.032
2-6	01/20/82	66	.102		.042		.027
2-7	02/15/82	70	.188		.068		.096
2-8	03/17/82	24	1.728		1.668		1.541
2-9	04/21/82	53	>3.00		2.443		>3.00

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TABLE 13 (continued)

ELISA RESULTS OBTAINED USING ANTI-IgG and ANTI-IgM MONOCLONAL CONJUGATE					
NANB ELISAs					
CASE #2					
2-10	05/19/82	95	>3.00	>3.00	>3.00
2-11	06/14/82	37	>3.00	>3.00	>1.00
CASE #3					
3-1	04/07/81	13	.193	.076	.049
3-2	05/12/81	236	.201	.051	.038
3-3	05/30/81	471	.132	.067	.052
3-4	06/09/81	315	.175	.155	.140
3-5	07/06/81	273	1.335	1.238	1.260
3-6	08/10/81	158	>3.00	>3.00	>3.00
3-7	09/08/81	84	>3.00	>3.00	>3.00
3-8	10/14/81	180	>3.00	>3.00	>3.00
3-9	11/11/81	154	>3.00	>3.00	>3.00

TABLE 14

ELISA RESULTS OBTAINED USING POLYCLONAL CONJUGATES								
NANB ELISAs								
			MONOCLONAL		TAGO	JACKSON		
			1:10K		1:80K		1:80K	
SAMPLE	DATE	ALT	OD	S/CO	OD	S/CO	OD	S/CO
NEG CONTROL			.076		.045		.154	
CUTOFF			.476		.545		.654	
PC (1:128)			1.390		.727		2.154	
CASE #1								
1-1	08/05/81	40	.178	.37	.067	.12	.153	.23
1-2	09/02/81	274	.154	.32	.097	.18	.225	.34
1-3	10/07/81	261	.129	.27	.026	.05	.167	.26
1-4	11/19/81	75	.937	1.97	.324	.60	.793	1.21
1-5	12/15/81	71	>3.00	>6.30	1.778	3.27	>3.00	>4.59
CASE #2								
2-1	10/19/81	17	.058	.12	.023	.04	.052	.08
2-2	11/17/81	46	.050	.11	.018	.03	.058	.09
2-3	12/02/81	63	.047	.10	.020	.04	.060	.09
2-4	12/14/81	152	.059	.12	.025	.05	.054	.08
2-5	12/23/81	624	.070	.15	.026	.05	.074	.11
2-6	01/20/82	66	.051	.11	.018	.03	.058	.09
2-7	02/15/82	70	.139	.29	.037	.07	.146	.22
2-8	03/17/82	24	1.867	3.92	.355	.65	1.429	2.19
2-9	04/21/82	53	>3.00	>6.30	.748	1.37	>3.00	>4.59
2-10	05/19/82	95	>3.00	>6.30	1.025	1.88	>3.00	>4.59
2-11	06/14/82	37	>3.00	>6.30	.917	1.68	>3.00	>4.59
CASE #3								
3-1	04/07/81	13	.090	.19	.049	.09	.138	.21

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TABLE 14 (continued)

ELISA RESULTS OBTAINED USING POLYCLONAL CONJUGATES								
			NANB ELISAs					
CASE #3								
3-2	05/12/81	236	.064	.13	.040	.07	.094	.14
3-3	05/30/81	471	.079	.17	.045	.08	.144	.22
3-4	06/09/81	315	.211	.44	.085	.16	.275	.42
3-5	07/06/81	273	1.707	3.59	.272	.50	1.773	2.71
3-6	08/10/81	158	>3.00	>6.30	1.347	2.47	>3.00	>4.59
3-7	09/08/81	84	>3.00	>6.30	2.294	4.21	>3.00	>4.59
3-8	10/14/81	180	>3.00	>6.30	>3.00	>5.50	>3.00	>4.59
3-9	11/11/81	154	>3.00	>6.30	>3.00	>5.50	>3.00	>4.59

## IV.I.6.b. Samples from Random Blood Donors

[0413] Samples from random blood donors (See Section IV.I.1.) were screened for HCV infection using the HCV c100-3 ELISA, in which the antibody-enzyme conjugate was either an anti-IgG monoclonal conjugate, or a polyclonal conjugate. The total number of samples screened were 1077 and 1056, for the polyclonal conjugate and the monoclonal conjugate, respectively. A summary of the results of the screening is shown in Table 15, and the sample distributions are shown in the histogram in Fig. 44.

[0414] The calculation of the average and standard deviation was performed excluding samples that gave a signal over 1.5, i.e., 1073 OD values were used for the calculations utilizing the polyclonal conjugate, and 1051 for the anti-IgG monoclonal conjugate. As seen in Table 15, when the polyclonal conjugate was used, the average was shifted from 0.0493 to 0.0931, and the standard deviation was increased from 0.074 to 0.0933. Moreover, the results also show that if the criteria of  $x + 5SD$  is employed to define the assay cutoff, the polyclonal-enzyme conjugate configuration in the ELISA requires a higher cutoff value. This indicates a reduced assay specificity as compared to the monoclonal system. In addition, as depicted in the histogram in Fig. 44, a greater separation of results between negative and positive distributions occurs when random blood donors are screened in an ELISA using the anti-IgG monoclonal conjugate as compared to the assay using a commercial polyclonal label.

TABLE 15

COMPARISON OF TWO ELISA CONFIGURATIONS IN TESTING SAMPLES FROM RANDOM BLOOD DONORS		
CONJUGATE	POLYCLONAL (Jackson)	ANTI-IgG MONOCLONAL
Number of samples	1073	1051
Average (x)	0.0931	0.04926
Standard deviation (SD)	0.0933	0.07427
5 SD	0.4666	0.3714
CUT-OFF (5 SD + x)	0.5596	0.4206

## IV.J. Detection of HCV Seroconversion in NANBH Patients from a Variety of Geographical Locations

[0415] Sera from patients who were suspected to have NANBH based upon elevated ALT levels, and who were negative in HAV and HBV tests were screened using the RIA essentially as described in Section IV.D., except that the HCV C100-3 antigen was used as the screening antigen in the microtiter plates. As seen from the results presented in Table 16, the RIA detected positive samples in a high percentage of the cases.

Table 16

Seroconversion Frequencies for Anti-c100-3 Among NANBH Patients in Different Countries			
Country	The Netherlands	Italy	Japan
No. Examined	5	36	26
No. Positive	3	29	19
% Positive	60	80	73

**EP 0 318 216 B2****IV.K. Detection of HCV Seroconversion in Patients with "Community Acquired" NANBH**

[0416] Sera which was obtained from 100 patients with NANBH, for whom there was no obvious transmission route (i.e., no transfusions, i.v. drug use, promiscuity, etc. were identified as risk factors), was provided by Dr. M. Alter of the Center for Disease Control, and Dr. J. Dlenstag of Harvard University. These samples were screened using an RIA essentially as described in Section IV.D., except that the HCV c100-3 antigen was used as the screening antigen attached to the microtiter plates. The results showed that of the 100 serum samples, 55 contained antibodies that reacted immunologically with the HCV c100-3 antigen.

[0417] The results described above suggest that "Community Acquired" NANBH is also caused by HCV. Moreover, since it has been demonstrated herein that HCV is related to Flaviviruses, most of which are transmitted by arthropods, it is suggestive that HCV transmission in the "Community Acquired" cases also results from arthropod transmission.

**IV.L. Comparison of Incidence of HCV Antibodies and Surrogate Markers In Donors Implicated in NANBH Transmission**

[0418] A prospective study was carried out to determine whether recipients of blood from suspected NANBH positive donors, who developed NANBH, seroconverted to anti-HCV-antibody positive. The blood donors were tested for the surrogate marker abnormalities which are currently used as markers for NANBH infection, i.e., elevated ALT levels, and the presence of anti-core antibody. In addition, the donors were also tested for the presence of anti-HCV antibodies. The determination of the presence of anti-HCV antibodies was determined using a radioimmunoassay as described in Section IV.K. The results of the study are presented in Table 17, which shows: the patient number (column 1); the presence of anti-HCV antibodies in patient serum (column 2); the number of donations received by the patient, with each donation being from a different donor (column 3); the presence of anti-HCV antibodies in donor serum (column 4); and the surrogate abnormality of the donor (column 5) (NT or -- means not tested) (ALT is elevated transaminase, and ANTI-HBc is anti-core antibody).

[0419] The results in Table 17 demonstrate that the HCV antibody test is more accurate in detecting infected blood donors than are the surrogate marker tests. Nine out of ten patients who developed NANBH symptoms tested positive for anti-HCV antibody seroconversion. Of the 11 suspected donors, (patient 6 received donations from two different individuals suspected of being NANBH carriers), 9 were positive for anti-HCV antibodies, and 1 was borderline positive, and therefore equivocal (donor for patient 1). In contrast, using the elevated ALT test 6 of the ten donors tested negative, and using the anticore-antibody test 5 of the ten donors tested negative. Of greater consequence, though, in three cases (donors to patients 8, 9, and 10) the ALT test and the ANTI-HBc test yielded inconsistent results.

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**Table 17**  
**DEVELOPMENT OF ANTI-HCV ANTIBODIES IN PATIENTS**  
**RECEIVING BLOOD FROM DONORS SUSPECTED OF BEING NANBH CARRIERS**

Patient	Anti-HCV Seroconversion in Patient	No. of Donations/Donors	Anti-HCV Positive Donor	Surrogate Abnormality Alt Anti-HB
1	yes	18	equiv	no
2	yes	18	yes	NT
3	yes	13	yes	no
4	no	18	no	--
5	yes	16	yes	yes
6	yes	11	yes(2)	no
7	yes	15	yes	yes
8	yes	20	yes	NT
9	yes	5	yes	no
10	yes	15	yes	yes

\*Same donor as anti-NANBV positive.

#### IV.M. Amplification for Cloning of HCV cDNA Sequences Utilizing the PCR and Primers Derived from Conserved Regions of Flavivirus Genomic Sequences

[0420] The results presented supra., which suggest that HCV is a flavivirus or flavi-like virus, allows a strategy for cloning uncharacterized HCV cDNA sequences utilizing the PCR technique, and primers derived from the regions encoding conserved amino acid sequences in flaviviruses. Generally, one of the primers is derived from a defined HCV genomic sequence, and the other primer which flanks a region of unsequenced HCV polynucleotide is derived from a conserved region of the flavivirus genome. The flavivirus genomes are known to contain conserved sequences within the NS1, and E polypeptides, which are encoded in the 5'-region of the flavivirus genome. Corresponding sequences encoding these regions lie upstream of the HCV cDNA sequence shown in Fig. 26. Thus, to isolate cDNA sequences derived from this region of the HCV genome, upstream primers are designed which are derived from the conserved sequences within these flavivirus polypeptides. The downstream primers are derived from an upstream end of the known portion of the HCV cDNA.

[0421] Because of the degeneracy of the code, it is probable that there will be mismatches between the flavivirus probes and the corresponding HCV genomic sequence. Therefore a strategy which is similar to the one described by

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Lee (1988) is used. The Lee procedure utilizes mixed oligonucleotide primers complementary to the reverse translation products of an amino acid sequence; the sequences in the mixed primers takes into account every codon degeneracy for the conserved amino acid sequence.

[0422] Three sets of primer mixes are generated, based on the amino acid homologies found in several flaviviruses, including Dengue-2,4 (D-2,4), Japanese Encephalitis Virus (JEV), Yellow Fever (YF), and West Nile Virus (WN). The primer mixture derived from the most upstream conserved sequence (5'-1), is based upon the amino acid sequence gly-trp-gly, which is part of the conserved sequence asp-arg-gly-trp-gly-aspN found in the E protein of D-2, JEV, YF, and WN. The next primer mixture (5'-2) is based upon a downstream conserved sequence in E protein, phe-asp-gly-asp-ser-tyr-ileu-phe-gly-asp-ser-tyr-ileu, and is derived from phe-gly-asp; the conserved sequence is present in D-2, JEV, YF, and WN. The third primer mixture (5'-3), is based on the amino acid sequence arg-ser-cys, which is part of the conserved sequence cys-cys-arg-ser-cys in the NS1 protein of D-2, D-4, JEV, YF, and WN. The individual primers which form the mixture in 5'-3 are shown in Fig. 45. In addition to the varied sequences derived from conserved region, each primer in each mixture also contains a constant region at the 5'-end which contains a sequence encoding sites for restriction enzymes, HindIII, MboI, and EcoRI.

[0423] The downstream primer, ssc5h20A, is derived from a nucleotide sequence in clone 5h, which contains HCV cDNA with sequences with overlap those in clones 14i and 11b. The sequence of ssc5h20A is

5' GTA ATA TGG TGA CAG AGT CA 3'.

An alternative primer, ssc5h34A, may also be used. This primer is derived from a sequence in clone 5h, and in addition contains nucleotides at the 5'-end which create a restriction enzyme site, thus facilitating cloning. The sequence of ssc5h34A is

5' GAT CTC TAG AGA AAT CAA TAT GGT GAC AGA GTC A 3'.

[0424] The PCR reaction, which was initially described by Saiki et al. (1986), is carried out essentially as described in Lee et al. (1988), except that the template for the cDNA is RNA isolated from HCV infected chimpanzee liver, as described in Section IV.C.2., or from viral particles isolated from HCV infected chimpanzee serum, as described in Section IV.A.1. In addition, the annealing conditions are less stringent in the first round of amplification (0.6M NaCl, and 25°C), since the part of the primer which will anneal to the HCV sequence is only 9 nucleotides, and there could be mismatches. Moreover, if ssc5h34A is used, the additional sequences not derived from the HCV genome tend to destabilize the primer-template hybrid. After the first round of amplification, the annealing conditions can be more stringent (0.066M NaCl, and 32 °C-37 °C), since the amplified sequences now contain regions which are complementary to, or duplicates of the primers. In addition, the first 10 cycles of amplification are run with Klenow enzyme I, under appropriate PCR conditions for that enzyme. After the completion of these cycles, the samples are extracted, and run with Taq polymerase, according to kit directions, as furnished by Cetus/Perkin-Elmer.

[0425] After the amplification, the amplified HCV cDNA sequences are detected by hybridization using a probe derived from clone 5h. This probe is derived from sequences upstream of those used to derive the primer, and does not overlap the sequences of the clone 5h derived primers. The sequence of the probe is

5' CCC AGC GGC GTA CGC GCT GGA CAC GGA GGT GGC CGC GTC  
GTG TGG CGG TGT TGT TCT CGT CGG GTT GAT GGC GC 3'.

#### IV.N.1. Creation of HCV cDNA Library from liver of a Chimpanzee with infectious NANBH

[0426] An HCV cDNA library was created from liver from the chimpanzee from which the HCV cDNA library in Section IV.A.1. was created. The technique for creating the library was similar to that in Section IV.A.24, except for this different source of the RNA, and that a primer based on the sequence of HCV cDNA in clone 11b was used. The sequence of the primer was

5' CTG GCT TGA AGA ATC 3'.

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IV.N.2. Isolation and nucleotide sequence of overlapping HCV cDNA in clone k9-1 to cDNA in clone 11b

[0427] Clone k9-1 was isolated from the HCV cDNA library created from the liver of an NANBH infected chimpanzee, as described in Section IV.A.25. The library was screened for clones which overlap the sequence in clone 11b, by using a clone which overlaps clone 11b at the 5'-terminus, clone 11a. The sequence of clone 11b is shown in Fig. 23. Positive clones were isolated with a frequency of 1 in 500,000. One isolated clone, k9-1, was subjected to further study. The overlapping nature of the HCV cDNA in clone k9-1, to the 5'-end of the HCV-cDNA sequence in Fig. 26 was confirmed by probing the clone with clone Alex 46; this latter clone contains an HCV cDNA sequence of 30 base pairs which corresponds to those base pairs at the 5'-terminus of the HCV cDNA in clone 14i, described supra..

[0428] The nucleotide sequence of the HCV cDNA isolated from clone k9-1 was determined using the techniques described supra. The sequence of the HCV cDNA in clone k9-1, the overlap with the HCV cDNA in Fig. 26, and the amino acids encoded therein are shown in Fig. 46.

[0429] The HCV cDNA sequence in clone k9-1 has been aligned with those of the clones described in Section IV.A. 19 to create a composite HCV cDNA sequence, with the k9-1 sequence being placed upstream of the sequence shown in Fig. 32. The composite HCV cDNA which includes the k9-1 sequence and the amino acids encoded therein is shown in Fig. 47.

[0430] The sequence of the amino acids encoded in the 5'-region of HCV cDNA shown in Fig. 47 has been compared with the corresponding region of one of the strains of Dengue virus, described supra., with respect to the profile of regions of hydrophobicity and hydrophilicity. This comparison showed that the polypeptides from HCV and Dengue encoded in this region, which corresponds to the region encoding NS1 (or a portion thereof), have a similar hydrophobic/hydrophilic profile.

[0431] The information provided infra. allows the identification of HCV strains. The isolation and characterization of other HCV strains may be accomplished by isolating the nucleic acids from body components which contain viral particles, creating cDNA libraries using polynucleotide probes based on the HCV cDNA probes described infra., screening the libraries for clones containing HCV cDNA sequences described infra., and comparing the HCV cDNAs from the new isolates with the cDNAs described infra. The polypeptides encoded therein, or in the viral genome, may be monitored for immunological cross-reactivity utilizing the polypeptides and antibodies described supra. Strains which fit within the parameters of HCV, as described in the Definitions section, supra., are readily identifiable. Other methods for identifying HCV strains will be obvious to those of skill in the art, based upon the information provided herein.

Industrial Applicability

[0432] The invention, in the various manifestations disclosed herein, has many industrial uses, some of which are the following. The HCV cDNAs may be used for the design of probes for the detection of HCV nucleic acids in samples. The probes derived from the cDNAs may be used to detect HCV nucleic acids in, for example, chemical synthetic reactions. They may also be used in screening programs for anti-viral agents, to determine the effect of the agents in inhibiting viral replication in cell culture systems, and animal model systems. The HCV polynucleotide probes are also useful in detecting viral nucleic acids in humans, and thus, may serve as a basis for diagnosis of HCV infections in humans.

[0433] In addition to the above, the cDNAs provided herein provide information and a means for synthesizing polypeptides containing epitopes of HCV. These polypeptides are useful in detecting antibodies to HCV antigens. A series of immunoassays for HCV infection, based on recombinant polypeptides containing HCV epitopes are described herein, and will find commercial use in diagnosing HCV induced NANBH, in screening blood bank donors for HCV-caused infectious hepatitis, and also for detecting contaminated blood from infectious blood donors. The viral antigens will also have utility in monitoring the efficacy of anti-viral agents in animal model systems. In addition, the polypeptides derived from the HCV cDNAs disclosed herein will have utility as vaccines for treatment of HCV infections.

[0434] The polypeptides derived from the HCV cDNAs, besides the above stated uses, are also useful for raising anti-HCV antibodies. Thus, they may be used in anti-HCV vaccines. However, the antibodies produced as a result of immunization with the HCV polypeptides are also useful in detecting the presence of viral antigens in samples. Thus, they may be used to assay the production of HCV polypeptides in chemical systems. The anti-HCV antibodies may also be used to monitor the efficacy of anti-viral agents in screening programs where these agents are tested in tissue culture systems. They may also be used for passive immunotherapy, and to diagnose HCV caused NANBH by allowing the detection of viral antigen(s) in both blood donors and recipients. Another important use for anti-HCV antibodies is in affinity chromatography for the purification of virus and viral polypeptides. The purified virus and viral polypeptide preparations may be used in vaccines. However, the purified virus may also be useful for the development of cell culture systems in which HCV replicates.

[0435] Cell culture systems containing HCV infected cells will have many uses. They can be used for the relatively large scale production of HCV, which is normally a low titer virus. These systems will also be useful for an elucidation

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of the molecular biology of the virus, and lead to the development of anti-viral agents. The cell culture systems will also be useful in screening for the efficacy of antiviral agents. In addition, HCV permissive cell culture systems are useful for the production of attenuated strains of HCV.

[0436] For convenience, the anti-HCV antibodies and HCV polypeptides, whether natural or recombinant, may be packaged into kits.

[0437] The method used for isolating HCV cDNA, which is comprised of preapring a cDNA library derived from infected tissue of an individual, in an expression vector, and selecting clones which produce the expression products which react immunologically with antibodies in antibody-containing body components from other infected individuals and not from non-infected individuals, may also be applicable to the isolation of cDNAs derived from other heretofore uncharacterized disease-associated agents which are comprised of a genomic component. This, in turn, could lead to isolation and characterization of these agents, and to diagnostic reagents and vaccines for these other disease-associated agents.

## Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, IT, LI, LU, NL, SE

1. A polymerase chain reaction (PCR) kit comprising a pair of primers capable of priming the synthesis of cDNA in a PCR reaction, wherein each of said primers is a polynucleotide comprising a contiguous sequence of nucleotides which is capable of selectively hybridizing to the genome of hepatitis C virus (HCV) or the complement thereof, wherein HCV is characterized by:

a positive stranded RNA genome;

said genome comprising an open reading frame (ORF) encoding a polyprotein; and

the entirety of the said encoded polyprotein having at least 40% homology to the entire polyprotein of a viral isolate from the genome of which was prepared cDNAs deposited in a lambda gt-11 cDNA library with the American Type Culture Collection (ATCC) under accession no. 40394.

2. A PCR kit according to claim 1 wherein each of said primers is a polynucleotide wherein said contiguous nucleotide seavorce is at least 20 nucleotides.

3. A PCR kit according to claim 1 or 2 further comprising a polynucleotide probe capable of selectively hybridising to a region of the HCV genome between and not including the HCV sequences from which the primers are derived.

4. A method of performing a polymerase chain reaction wherein the primers are a pair of polynucleotides as defined in claim 1 or 2.

5. A method for assaying a sample for the presence or absence of HCV polynucleotides comprising:

(a) contacting the sample with a probe under conditions that allow the selective hybridisation of said probe to an HCV polynucleotide or the complement thereof in the sample, wherein said probe comprises a polynucleotide comprising a contiguous sequence of nucleotides which is capable of selectively hybridising to the genome of HCV or the complement thereof, wherein HCV is characterised by:

(i) a positive stranded RNA genome, said genome comprising an open reading frame (ORF) encoding a polyprotein; and

(ii) the entirety of the said encoded polyprotein having at least 40% homology to the entire polyprotein of a viral isolate from the genome of which was prepared cDNAs deposited in a lambda gt-11 cDNA library with the American Type Culture Collection (ATCC) under accession no. 40394;

and

(b) determining whether polynucleotide duplexes comprising said probe are formed,

and further wherein said polynucleotide is a DNA polynucleotide and optionally comprises a detectable label.

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### Claims for the following Contracting State : ES

1. A method of performing a polymerase chain reaction wherein the primers are a pair of polynucleotides, which polynucleotides are each a contiguous sequence of nucleotides which is capable of selectively hybridizing to the genome of hepatitis C virus (HCV) or the complement thereof, wherein HCV is characterized by:
  - (i) a positive stranded RNA genome;
  - (ii) said genome comprising an open reading frame (ORF) encoding a polyprotein; and
  - (iii) the entirety of the said polyprotein having at least 40% homology to the entire polyprotein of a viral isolate from the genome of which was prepared cDNAs deposited in a lambda gt-11 cDNA library with the American Type Culture Collection (ATCC) under accession no. 40394.
2. A method according to claim 1 wherein the entirety of said polyprotein has at least 60% homology to the entire polyprotein.
3. A method according to claim 1 or 2 wherein said contiguous sequence is at least 10 nucleotides.
4. A method according to claim 3 wherein said contiguous sequence is at least 15 nucleotides.
5. A method according to claim 4 wherein said contiguous sequence is at least 20 nucleotides.
6. A method according to any one of claims 1 to 5 wherein each polynucleotide is a DNA polynucleotide.
7. A method according to any one of claims 1 to 6 further comprising the use of a polynucleotide probe capable of selectively hybridising to a region of the HCV genome between and not including the HCV sequences from which the primers are derived.
8. A method for assaying a sample for the presence or absence of HCV polynucleotides comprising:
  - (a) contacting the sample with a probe under conditions that allow the selective hybridisation of said polynucleotide to an HCV polynucleotide or the complement thereof in the sample wherein said probe comprises a polynucleotide comprising a contiguous sequence of nucleotides which is capable of selectively hybridising to the genome of HCV or the complement thereof, wherein HCV is characterised by:
    - (i) a positive stranded RNA genome, said genome comprising an open reading frame (ORF) encoding a polyprotein; and
    - (ii) the entirety of the said encoded polyprotein having at least 40% homology to the entire polyprotein of a viral isolate from the genome of which was prepared cDNAs deposited in a lambda gt-11 cDNA library with the American Type Culture Collection (ATCC) under accession no. 40394
  - and
  - (b) determining whether polynucleotide duplexes comprising said polynucleotide are formed,and further wherein the probe further comprises a detectable label.
9. A method according claim 8 wherein the probe is fixed to a solid phase.

### Claims for the following Contracting State : GR

1. A method of performing a polymerase chain reaction wherein the primers are a pair of polynucleotides, which polynucleotides are each a contiguous sequence of nucleotides which is capable of selectively hybridizing to the genome of hepatitis C virus (HCV) or the complement thereof, wherein HCV is characterized by:
  - (i) a positive stranded RNA genome;
  - (ii) said genome comprising an open reading frame (ORF) encoding a polyprotein; and
  - (iii) the entirety of the said polyprotein having at least 40% homology to the entire polyprotein of a viral isolate from the genome of which was prepared cDNAs deposited in a lambda gt-11 cDNA library with the American

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Type Culture Collection (ATCC) under accession no.40394.

2. A method according to claim 1 wherein the entirety of said polypeptide has at least 60% homology to the entire polypeptide.

3. A method according to claim 1 or 2 wherein said contiguous sequence is at least 10 nucleotides.

4. A method according to claim 3 wherein said contiguous sequence is at least 15 nucleotides.

5. A method according to claim 4 wherein said contiguous sequence is at least 20 nucleotides.

6. A method according to any one of claims 1 to 5 wherein each polynucleotide is a DNA polynucleotide.

7. A method according to any one of claims 1 to 6 further comprising the use of a polynucleotide probe capable of selectively hybridising to a region of the HCV genome between and not including the HCV sequences from which the primers are derived.

8. A method for assaying a sample for the presence or absence of HCV polynucleotides comprising:

(a) contacting the sample with a probe under conditions that allow the selective hybridisation of said polynucleotide to an HCV polynucleotide or the complement thereof in the sample wherein said probe comprises a polynucleotide comprising a contiguous sequence of nucleotides which is capable of selectively hybridising to the genome of HCV or the complement thereof, wherein HCV is characterised by:

(i) a positive stranded RNA genome, said genome comprising an open reading frame (ORF) encoding a polypeptide; and

(ii) the entirety of the said encoded polypeptide having at least 40% homology to the entire polypeptide of a viral isolate from the genome of which was prepared cDNAs deposited in a lambda gt-11 cDNA library with the American Type Culture Collection (ATCC) under accession no. 40394

and

(b) determining whether polynucleotide duplexes comprising said polynucleotide are formed

and further wherein the probe further comprises a detectable label.

9. A method according claim 8 wherein the probe is fixed to a solid phase.

10. A polymerase chain reaction (PCR) kit comprising a pair of primers capable of priming the synthesis of cDNA in a PCR reaction, wherein each of said primers is a polynucleotide comprising a contiguous sequence of nucleotides which is capable of selectively hybridizing to the genome of hepatitis C virus (HCV) or the complement thereof, wherein HCV is characterized by:

(i) a positive stranded RNA genome;

(ii) said genome comprising an open reading frame (ORF) encoding a polypeptide; and

(iii) the entirety of the said polypeptide having at least 40% homology to the entire polypeptide of a viral isolate from the genome of which was prepared cDNAs deposited in a lambda gt-11 cDNA library with the American Type Culture Collection (ATCC) under accession no. 40394.

11. A PCR kit according to claim 10 wherein each of the primers is a DNA polynucleotide.

12. A PCR kit according to claim 10 or 11 further comprising a polynucleotide probe capable of selectively hybridising to a region of the HCV genome between and not including the HCV sequences from which the primers are derived.

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## Patentansprüche

## Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, IT, LI, LU, NL, SE

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1. Polymerasekettenreaktions (PCR)-Kit, umfassend ein Paar von Primern, die zum Primen der Synthese von cDNA in einer PCR-Reaktion fähig sind, wobei jeder der Primer ein Polynucleotid ist, das eine benachbarte Sequenz von Nucleotiden umfasst, die zur selektiven Hybridisierung mit dem Genom von Hepatitis C-Virus (HCV) oder dem Komplement davon fähig ist, wobei HCV charakterisiert ist:

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durch ein Plusstrang-RNA-Genom;

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wobei das Genom ein offenes Leseraster (ORF) umfasst, welches ein Polyprotein codiert; und dadurch daß die Gesamtheit des codierten Polyproteins eine mindestens 40%-ige Homologie zu dem gesamten Polyprotein eines viralen Isolats hat, von dessen Genom cDNAs hergestellt wurden, hinterlegt in einer Lambda-gt-11-cDNA-Bibliothek bei der American Type Culture Collection (ATCC) unter der Hinterlegungsnummer 40394.

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2. PCR-Kit nach Anspruch 1, wobei jeder der Primer ein Polynucleotid ist, wobei die benachbarte Nucleotidsequenz mindestens 20 Nucleotide umfasst.

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3. PCR-Kit nach Anspruch 1 oder 2, weiterhin eine Polynucleotidsonde umfassend, die zur selektiven Hybridisierung mit einem Bereich des HCV-Genoms fähig ist, das zwischen den HCV-Sequenzen liegt, von denen die Primer stammen, und diese nicht umfasst.

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4. Verfahren zur Durchführung einer Polymerasekettenreaktion, wobei die Primer wie in Anspruch 1 oder 2 definiert sind.

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5. Verfahren zum Testen einer Probe auf die Gegenwart oder Abwesenheit von HCV-Polynucleotiden, umfassend:

(a) Inkontaktbringen der Probe mit einer Sonde unter Bedingungen, die die selektive Hybridisierung der Sonde mit einem HCV-Polynucleotid oder dem Komplement davon in der Probe ermöglichen, wobei die Sonde ein Polynucleotid umfasst, das eine benachbarte Sequenz von Nucleotiden umfasst, die fähig ist zur selektiven Hybridisierung mit dem Genom von HCV oder dem Komplement davon, wobei HCV charakterisiert ist:

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- (i) durch ein Plusstrang-RNA-Genom, wobei das Genom ein offenes Leseraster (ORF) umfasst, welches ein Polyprotein codiert; und
- (ii) dadurch, daß die Gesamtheit des codierten Polyproteins eine mindestens 40%-ige Homologie zu dem gesamten Polyprotein eines viralen Isolats hat, von dessen Genom cDNAs hergestellt wurden, hinterlegt in einer Lambda-gt-11-cDNA-Bibliothek bei der American Type Culture Collection (ATCC) unter der Hinterlegungsnummer 40394;

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und

(b) Bestimmung, ob Polynucleotidduplexe gebildet wurden, die die Sonde umfassen,

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und wobei weiterhin das Polynucleotid ein DNA-Polynucleotid ist und gegebenenfalls einen nachweisbaren Marker umfasst.

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## Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Durchführung einer Polymerasekettenreaktion, wobei die Primer ein Paar von Polynucleotiden sind, wobei die Polynucleotide jeweils eine benachbarte Sequenz von Nucleotiden umfassen, die zur selektiven Hybridisierung mit dem Genom von Hepatitis C-Virus (HCV) oder dem Komplement davon fähig ist, wobei HCV charakterisiert ist:

60

- (i) durch ein Plusstrang-RNA-Genom;
- (ii) wobei das Genom ein offenes Leseraster (ORF) umfasst, welches ein Polyprotein codiert; und

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(III) dadurch, daß die Gesamtheit des Polyproteins eine mindestens 40%-ige Homologie zu dem gesamten Polyprotein eines viralen Isolats hat, von dessen Genom cDNAs hergestellt wurden, hinterlegt in einer Lambda-gt-11-cDNA-Bibliothek bei der American-Type Culture Collection (ATCC) unter der Hinterlegungsnummer 40394.

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2. Verfahren nach Anspruch 1, wobei die Gesamtheit des Polyproteins mindestens 60 % Homologie mit dem gesamten Polyprotein aufweist.

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3. Verfahren nach Anspruch 1 oder 2, wobei die benachbarte Sequenz mindestens 10 Nucleotide umfasst.

4. Verfahren nach Anspruch 3, wobei die benachbarte Sequenz mindestens 15 Nucleotide umfasst.

5. Verfahren nach Anspruch 4, wobei die benachbarte Sequenz mindestens 20 Nucleotide umfasst.

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6. Verfahren nach einem der Ansprüche 1 bis 5, wobei jedes Polynucleotid ein DNA-Polynucleotid ist.

7. Verfahren nach einem der Ansprüche 1 bis 6, weiterhin umfassend die Verwendung einer Polynucleotidsonde, die zur selektiven Hybridisierung mit einem Bereich des HCV-Genoms fähig ist, das zwischen den HCV-Sequenzen liegt, von denen die Primer stammen, und diese nicht umfasst.

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8. Verfahren zum Testen einer Probe auf die Gegenwart oder Abwesenheit von HCV-Polynucleotiden, umfassend:

25

(a) Inkontaktbringen der Probe mit einer Sonde unter Bedingungen, die die selektive Hybridisierung des Polynucleotids mit einem HCV-Polynucleotid oder dem Komplement davon in der Probe ermöglichen, wobei die Sonde ein Polynucleotid umfasst, das eine benachbarte Sequenz von Nucleotiden umfasst, die zur selektiven Hybridisierung mit dem Genom von HCV oder dem Komplement davon fähig ist, wobei HCV charakterisiert ist:

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(i) durch ein Plusstrang-RNA-Genom, wobei das Genom ein offenes Leseraster (ORF) umfasst, welches ein Polyprotein codiert; und

(ii) dadurch, daß die Gesamtheit des codierten Polyproteins eine mindestens 40%-ige Homologie zu dem gesamten Polyprotein eines viralen Isolats hat, von dessen Genom cDNAs hergestellt wurden, hinterlegt in einer Lambda-gt-11-cDNA-Bibliothek bei der American Type Culture Collection (ATCC) unter der Hinterlegungsnummer 40394; und

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(b) Bestimmung, ob Polynucleotidduplexe gebildet wurden, die diese Polynucleotide umfassen,

und wobei die Sonde ferner einen nachweisbaren Marker umfasst.

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9. Verfahren nach Anspruch 8, wobei die Sonde an eine feste Phase fixiert ist.

#### Patentansprüche für folgenden Vertragsstaat : GR

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1. Verfahren zur Durchführung einer Polymerasekettenreaktion, wobei die Primer ein Paar von Polynucleotiden sind, wobei die Polynucleotide jeweils eine benachbarte Sequenz von Nucleotiden umfassen, die zur selektiven Hybridisierung mit dem Genom von Hepatitis C-Virus (HCV) oder dem Komplement davon fähig ist, wobei HCV charakterisiert ist:

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(I) durch ein Plusstrang-RNA-Genom;

(II) wobei das Genom ein offenes Leseraster (ORF) umfasst, welches ein Polyprotein codiert; und

(III) dadurch, daß die Gesamtheit des codierten Polyproteins eine mindestens 40%-ige Homologie zu dem gesamten Polyprotein eines viralen Isolats hat, von dessen Genom cDNAs hergestellt wurden, hinterlegt in einer Lambda-gt-11-cDNA-Bibliothek bei der American Type Culture Collection (ATCC) unter der Hinterlegungsnummer 40394.

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2. Verfahren nach Anspruch 1, wobei die Gesamtheit des Polyproteins 60% Homologie mit dem gesamten Polyprotein aufweist.

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3. Verfahren nach Anspruch 1 oder 2, wobei die benachbarte Sequenz mindestens 10 Nucleotide umfasst.
4. Verfahren nach Anspruch 3, wobei die benachbarte Sequenz mindestens 15 Nucleotide umfasst.
5. Verfahren nach Anspruch 4, wobei die benachbarte Sequenz mindestens 20 Nucleotide umfasst.
6. Verfahren nach einem der Ansprüche 1 bis 5, wobei jedes Polynucleotid ein DNA-Polynucleotid ist.
7. Verfahren nach einem der Ansprüche 1 bis 6, weiterhin umfassend die Verwendung einer Polynucleotidsonde, die zur selektiven Hybridisierung mit einem Bereich des HCV-Genoms fähig ist, der zwischen den HCV-Sequenzen liegt, von denen die Primer stammen, und diese nicht umfasst.
8. Verfahren zum Testen einer Probe auf die Gegenwart oder Abwesenheit von HCV-Polynucleotiden, umfassend:
  - (a) Inkontaktbringen der Probe mit einer Sonde unter Bedingungen, die die selektive Hybridisierung des Polynucleotids mit einem HCV-Polynucleotid oder dem Komplement davon in der Probe ermöglichen, wobei die Sonde ein Polynucleotid umfasst, das eine benachbarte Sequenz von Nucleotiden umfasst, die zur selektiven Hybridisierung mit dem Genom von HCV oder dem Komplement davon fähig ist, wobei HCV charakterisiert ist:
    - (i) durch ein Plusstrang-RNA-Genom, wobei das Genom ein offenes Leseraster (ORF) umfasst, welches ein Polyprotein codiert; und
    - (ii) dadurch, daß die Gesamtheit des codierten Polyproteins eine mindestens 40%-ige Homologie zu dem gesamten Polyprotein eines viralen Isolats hat, von dessen Genom cDNAs hergestellt wurden, hinterlegt in einer Lambda-gt-11-cDNA-Bibliothek bei der American Type Culture Collection (ATCC) unter der Hinterlegungsnummer 40394; und
  - (b) Bestimmung, ob Polynucleotidduplexe gebildet wurden, die die Polynucleotide umfassen, und wobei ferner die Sonde einen nachweisbaren Marker umfasst.
9. Verfahren nach Anspruch 8, wobei die Sonde an eine feste Phase fixiert ist.
10. Polymerasekettenreaktions (PCR)-Kit, umfassend ein Paar von Primern, die zum Primen der Synthese von cDNA in einer PCR-Reaktion fähig sind, wobei jeder der Primer ein Polynucleotid ist, das eine benachbarte Sequenz von Nucleotiden umfasst, die zur selektiven Hybridisierung mit dem Genom von Hepatitis C-Virus (HCV) oder dem Komplement davon fähig ist, wobei HCV charakterisiert ist:
  - (i) durch ein Plusstrang-RNA-Genom;
  - (ii) wobei das Genom ein offenes Leseraster (ORF) umfasst, welches ein Polyprotein codiert; und
  - (iii) dadurch, daß die Gesamtheit des codierten Polyproteins eine mindestens 40%-ige Homologie zu dem gesamten Polyprotein eines viralen Isolats hat, von dessen Genom cDNAs hergestellt wurden, hinterlegt in einer Lambda-gt-11-cDNA-Bibliothek bei der American Type Culture Collection (ATCC) unter der Hinterlegungsnummer 40394.
11. PCR-Kit nach Anspruch 10, wobei jeder Primer ein DNA-Polynucleotid ist.
12. PCR-Kit nach Anspruch 10 oder 11, weiterhin eine Polynucleotidsonde umfassend, die zur selektiven Hybridisierung mit einem Bereich des HCV-Genoms fähig ist, der zwischen den HCV-Sequenzen liegt, von denen die Primer stammen, und diese nicht umfasst.

**Revendications**

**Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, IT, LI, LU, NL, SE**

1. *Nécessaire pour réaction d'amplification en chaîne par polymérase (PCR), comprenant une paire d'amorces capables d'amorcer la synthèse d'un ADNc dans une réaction PCR, dans lequel chacune desdites amorces est un*

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polynucléotide comprenant une séquence contiguë de nucléotides qui est capable de s'hybrider sélectivement avec le génome du virus de l'hépatite C (HCV) ou son complément, le HCV étant caractérisé par

- un génome à ARN à brin positif ;
  - le fait que ledit génome comprend un cadre de lecture ouvert (ORF) codant pour une polyprotéine; et
  - le fait que l'intégralité de ladite polyprotéine a une homologie d'au moins 40 % avec l'entière polyprotéine d'un isolat viral à partir du génome duquel ont été préparés les ADNc déposés dans une banque d'ADNc dans lambda gt-11 à l'American Type Culture Collection (ATCC) sous le n° de dépôt 40394.
2. Nécessaire pour PCR selon la revendication 1, dans lequel chacune desdites amorces est un polynucléotide dans lequel ladite séquence contiguë de nucléotides comporte au moins 20 nucléotides.
3. Nécessaire pour PCR selon la revendication 1 ou 2, comprenant en outre une sonde polynucléotidique capable de s'hybrider sélectivement avec une région du génome du HCV située entre et n'incluant pas les séquences de HCV desquelles sont issues les amorces.
4. Procédé d'exécution d'une réaction d'amplification en chaîne par polymérase, dans lequel les amorces consistent en une paire de polynucléotides tels que définis dans la revendication 1 ou 2.
5. Procédé pour l'essai d'un échantillon pour la détermination de la présence ou de l'absence de polynucléotides de HCV, comprenant:
- (a) la mise en contact de l'échantillon avec une sonde dans des conditions permettant l'hybridation sélective de ladite sonde avec un polynucléotide de HCV ou son complément dans l'échantillon, ladite sonde comprenant un polynucléotide comprenant une séquence contiguë de nucléotides qui est capable de s'hybrider sélectivement avec le génome de HSV ou son complément, le HCV étant caractérisé par :
- (I) un génome à ARN à brin positif, ledit génome comprenant un cadre de lecture ouvert (ORF) codant pour une polyprotéine ; et
- (II) le fait que l'intégralité de ladite polyprotéine codée a une homologie d'au moins 40 % avec l'entière polyprotéine d'un isolat viral à partir du génome duquel ont été préparés les ADNc déposés dans une banque d'ADNc dans lambda gt-11 à l'American Type Culture Collection (ATCC) sous le n° de dépôt 40394 ;
- et
- (b) l'étape consistant à déterminer si des duplex polynucléotidiques comprenant ladite sonde se sont formés ; et en outre dans lequel ledit polynucléotide est un polynucléotide d'ADN et comprend éventuellement un marqueur détectable.

**Revendications pour l'Etat contractant suivant : ES**

1. Procédé d'exécution d'une réaction d'amplification en chaîne par polymérase, dans lequel les amorces sont une paire de polynucléotides, lesquels polynucléotides sont chacun une séquence contiguë de nucléotides qui est capable de s'hybrider sélectivement avec le génome du virus de l'hépatite C (HCV) ou son complément, le HCV étant caractérisé par
- (I) un génome à ARN à brin positif ;
- (II) le fait que ledit génome comprend un cadre de lecture ouvert (ORF) codant pour une polyprotéine; et
- (III) le fait que l'intégralité de ladite polyprotéine a une homologie d'au moins 40 % avec l'entière polyprotéine d'un isolat viral à partir du génome duquel ont été préparés les ADNc déposés dans une banque d'ADNc dans lambda gt-11 à l'American Type Culture Collection (ATCC) sous le n° de dépôt 40394.
2. Procédé selon la revendication 1, dans lequel l'intégralité de ladite polyprotéine a une homologie d'au moins 60 % avec l'entière polyprotéine.
3. Procédé selon la revendication 1 ou 2, dans lequel ladite séquence contiguë comporte au moins 10 nucléotides.

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4. Procédé selon la revendication 3, dans lequel ladite séquence contiguë comporte au moins 15 nucléotides.
5. Procédé selon la revendication 4, dans lequel ladite séquence contiguë comporte au moins 20 nucléotides.
- 5 6. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel chaque polynucléotide est un polynucléotide d'ADN.
7. Procédé selon l'une quelconque des revendications 1 à 6, qui comprend en outre l'utilisation d'une sonde polynucléotidique capable de s'hybrider sélectivement avec une région du génome du HCV située entre et n'incluant pas  
10 les séquences de HCV desquelles sont issues les amorces.
8. Procédé pour l'essai d'un échantillon pour la détermination de la présence ou de l'absence de polynucléotides de HCV, comprenant:  
15 (a) la mise en contact de l'échantillon avec une sonde dans des conditions permettant l'hybridation sélective dudit polynucléotide avec un polynucléotide de HCV ou son complément dans l'échantillon, ladite sonde comprenant un polynucléotide comprenant une séquence contiguë de nucléotides qui est capable de s'hybrider sélectivement avec le génome de HSV ou son complément, le HCV étant caractérisé par :  
20 (I) un génome à ARN à brin positif, ledit génome comprenant un cadre de lecture ouvert (ORF) codant pour une polyprotéine ; et  
(II) le fait que l'intégralité de ladite polyprotéine codée a une homologie d'au moins 40 % avec l'entière polyprotéine d'un isolat viral à partir du génome duquel ont été préparés les ADNc déposés dans une banque d'ADNc dans lambda gt-11 à l'American Type Culture Collection (ATCC) sous le n° de dépôt  
25 40394 ;  
et  
(b) l'étape consistant à déterminer si des duplex polynucléotidiques comprenant ledit polynucléotide se sont formés ;  
30 et en outre dans lequel la sonde comprend en outre un marqueur détectable.
9. Procédé selon la revendication 8, dans lequel la sonde est fixée sur une phase solide.
- 35 **Revendications pour l'Etat contractant suivant : GR**
1. Procédé d'exécution d'une réaction d'amplification en chaîne par polymérase, dans lequel les amorces sont une paire de polynucléotides, lesquels polynucléotides sont chacun une séquence contiguë de nucléotides qui est capable de s'hybrider sélectivement avec le génome du virus de l'hépatite C (HCV) ou son complément, le HCV étant caractérisé par  
40 (I) un génome à ARN à brin positif ;  
(II) le fait que ledit génome comprend un cadre de lecture ouvert (ORF) codant pour une polyprotéine ; et  
(III) le fait que l'intégralité de ladite polyprotéine a une homologie d'au moins 40 % avec l'entière polyprotéine d'un isolat viral à partir du génome duquel ont été préparés les ADNc déposés dans une banque d'ADNc dans lambda gt-11 à l'American Type Culture Collection (ATCC) sous le n° de dépôt 40394.  
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2. Procédé selon la revendication 1, dans lequel l'intégralité de ladite polyprotéine a une homologie d'au moins 60 % avec l'entière polyprotéine.  
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3. Procédé selon la revendication 1 ou 2, dans lequel ladite séquence contiguë comporte au moins 10 nucléotides.
4. Procédé selon la revendication 3, dans lequel ladite séquence contiguë comporte au moins 15 nucléotides.  
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5. Procédé selon la revendication 4, dans lequel ladite séquence contiguë comporte au moins 20 nucléotides.
6. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel chaque polynucléotide est un polynucléotide

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d'ADN.

7. Procédé selon l'une quelconque des revendications 1 à 6, qui comprend en outre l'utilisation d'une sonde polynucléotidique capable de s'hybrider sélectivement avec une région du génome du HCV située entre et n'incluant pas les séquences de HCV desquelles sont issues les amorces.
8. Procédé pour l'essai d'un échantillon pour la détermination de la présence ou de l'absence de polynucléotides de HCV, comprenant:
  - (a) la mise en contact de l'échantillon avec une sonde dans des conditions permettant l'hybridation sélective dudit polynucléotide avec un polynucléotide de HCV ou son complément dans l'échantillon, ladite sonde comprenant un polynucléotide comprenant une séquence contiguë de nucléotides qui est capable de s'hybrider avec le génome de HCV ou son complément, le HCV étant caractérisé par :
    - (I) un génome à ARN à brin positif, ledit génome comprenant un cadre de lecture ouvert (ORF) codant pour une polyprotéine ; et
    - (II) le fait que l'intégralité de ladite polyprotéine codée a une homologie d'au moins 40 % avec l'entière polyprotéine d'un isolat viral à partir du génome duquel ont été préparés les ADNc déposés dans une banque d'ADNc dans lambda gt-11 à l'American Type Culture Collection (ATCC) sous le n° de dépôt 40394 ;
  - et
  - (b) l'étape consistant à déterminer si des duplex polynucléotidiques comprenant ledit polynucléotide se sont formés ; et en outre dans lequel la sonde comprend en outre un marqueur détectable.
9. Procédé selon la revendication 8, dans lequel la sonde est fixée sur une phase solide.
10. Nécessaire pour réaction d'amplification en chaîne par polymérase (PCR), comprenant une paire d'amorces capable d'amorcer la synthèse d'un ADNc dans une réaction PCR, dans lequel chacune desdites amorces est un polynucléotide comprenant une séquence contiguë de nucléotides qui est capable de s'hybrider sélectivement avec le génome du virus de l'hépatite C (HCV) ou son complément, le HCV étant caractérisé par
  - (I) un génome à ARN à brin positif ;
  - (II) le fait que ledit génome comprend un cadre de lecture ouvert (ORF) codant pour une polyprotéine; et
  - (III) le fait que l'intégralité de ladite polyprotéine a une homologie d'au moins 40 % avec l'entière polyprotéine d'un isolat viral à partir du génome duquel ont été préparés les ADNc déposés dans une banque d'ADNc dans lambda gt-11 à l'American Type Culture Collection (ATCC) sous le n° de dépôt 40394.
11. Nécessaire pour PCR selon la revendication 10, dans lequel chacune des amorces est un polynucléotide d'ADN.
12. Nécessaire pour PCR selon la revendication 10 ou 11, comprenant en outre une sonde polynucléotidique capable de s'hybrider sélectivement avec une région du génome de HCV située entre et n'incluant pas les séquences de HCV desquelles sont issues les amorces.

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FIG. 1 Translation of DNA 5-1-1

AlaSerCysLeuAsnCysSerAlaSerIleIleProAspArgGluValLeuTyrArgGlu  
 1 GGCCTCCTGCTTGAACCTGCTCGGCGAGCATCATACCTGACAGGGAAGTCCTCTACCGAGA  
 CCGGAGGACGAACCTTGACGAGCCGCTCGTAGTATGGACTGTCCCTTCAGGAGATGGCTCT

PheAspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeu  
 61 GTTCGATGAGATGGAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCT  
 CAAGCTACTCTACCTTCTCAGGAGAGTCGTGAATGGCATGTAGCTCGTTCCCTACTACGA

AlaGluGlnPheLysGlnLysAlaLeuGlyLeu  
 121 CGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCC  
 GCGGCTCGTCAAGTTCGTCTCCGGGAGCCGGAGG

FIG. 3 Translation of DNA 5-1-1, 81, 91&amp;1-2

GlyCysValValIleValGlyArgValValLeuSerGlyLysProAlaIleIleProAsp  
 1 CTGGCTGCGTGGTCATAGTGGGCAGGTCGTCTTGTCCGGGAAGCCGGCAATCATACTG  
 GACCGACGCACCACTATCACCCGTCCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGAC

T

ArgGluValLeuTyrArgGluPheAspGluMetGluGluCysSerGlnHisLeuProTyr  
 61 ACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATGGAAGAGTGCTCTCAGCACTTACCGT  
 TGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTCTCAGGAGAGTCGTGAATGGCA

A

IleGluGlnGlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeuGln  
 121 ACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCCTGC  
 TGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCGTCTTCGGGAGCCGGAGGACG

ThrAlaSerArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLysLeu  
 181 AGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCTGCTGTCCAGACCAACTGGCAAAAAC  
 TCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGACGACAGGTCTGGTTGACCGTTTTTG

GluThrPheTrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAlaGly  
 241 TCGAGACCTTCTGGGCGAAGCATATGTGGAACCTTCATCAGTGGGATACAATACTTGGCGG  
 AGCTCTGGAAGACCGCTTCGTATACACCTGAAGTAGTCACCTATGTTATGAACCGCC

LeuSerThrLeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAlaVal  
 301 GCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACAGCTGCTG  
 CGAACAGTTGCGACGGACCATTTGGGGCGGTAACTAAGTAAGTACCGAAAATGTCGACGAC

ThrSerProLeuThrThrSerGln  
 361 TCACCAGCCCACTAACCCTAGCCAAA  
 AGTGGTCGGGTGATTGGTGATCGGTTT

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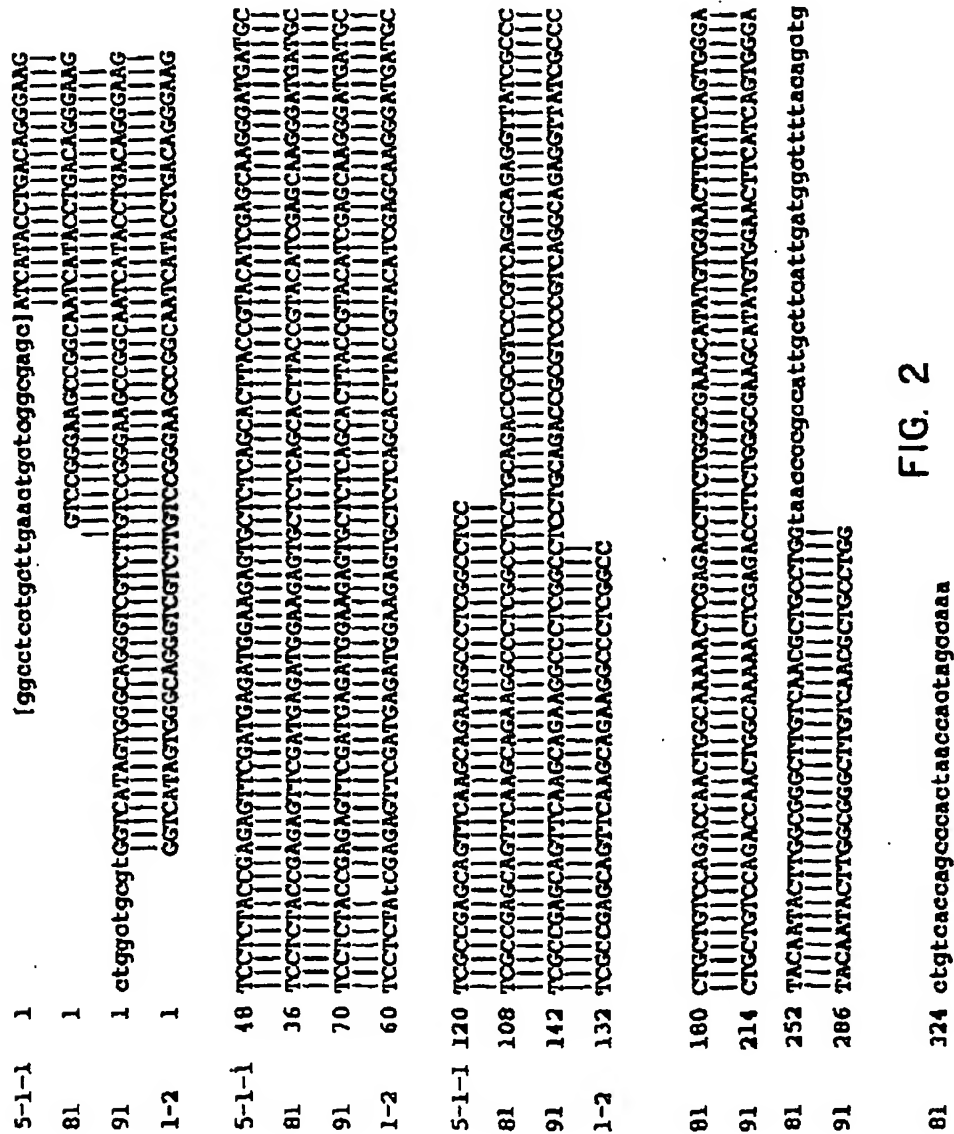


FIG. 2

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FIG. 4 Translation of DNA 81

1 SerGlyLysProAlaIleIleProAspArgGluValLeuTyrArgGluPheAspGluMet  
GTCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTCCCTTACCGAGAGTTCGATGAGAT  
CAGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTA

61 GluGluCysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPhe  
GGAAGAGTGTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTT  
CCTTCTCAGGAGTCTGTAATGGCATGTAGCTCGTTCCTACTACGAGCGGCTCGTCAA

121 LysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaPro  
CAAGCAGAAGGCCCTCGGCCCTCTGACAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCC  
GTTCTGCTTCCGGGAGCCGGAGGACGCTGCGCGAGGGCAGTCCGCTCCAATAGCGGGG

181 AlaValGlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPhe  
TGCTGTCCAGACCAACTGGCAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAAGTT  
ACGACAGGTCTGGTTGACCGTTTTTGAGCTCTGGAAGACCGCTTCGTATACACCTTGAA

241 IleSerGlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAla  
CATCAGTGGGATACAATACTTGGCGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGC  
GTAGTCACCTATGTTATGAACCGCCCGAACAGTTGCGACGGACCATGGGGCGGTAACG

301 SerLeuMetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGln  
TTCATTGATGGCTTTTACAGCTGCTGTCAACAGCCCACTAACCACCTAGCCAAA  
AAGTAACCTACCGAAAATGTCGACGACAGTGGTGGGTGATTGGTGATCGGTTT

FIG. 5 Translation of DNA 36

1 AspAlaHisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAla  
GATGCCCACTTTCTATCCAGACAAAGCAGAGTGGGAGAACCTTCCTTACCTGGTAGCG  
CTACGGGTGAAAGATAGGGTCTGTTCTCTCACCCCTCTTGAAGGAATGGACCATCGC

61 TyrGlnAlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrp  
TACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCTCCCCATCGTGGGACCAGATGTGG  
ATGGTTCCGTGGCACACGCGATCCCGAGTTCGGGGAGGGGTAGCACCTGGTCTACACC

121 LysCysLeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeu  
AAGTGTGTTGATTGCGCTCAAGCCCACTCCATGGGCCAACACCCCTGCTATACAGACTG  
TTCACAACTAAGCGAGTTCGGGTGGGAGGTACCGGTTGTGGGGACGATATGTCTGAC

181 GlyAlaValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIleMetThrCys  
GGCGCTGTTCAGAAATGAAATCACCTGACGCACCCAGTCACCAAATACATCATGACATGC  
CCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGGTGAGTGGTTTATGTAGTACTGTACC

241 MetSerAlaAspLeuGluValValThrSerThrTrpValLeuValGlyGlyValLeuAla  
ATGTCCGGCGACCTGGAGGTGCTCAGCAGCACCTGGGTGCTCGTTGGCGGCGTCTGGCT  
TACAGCCGCTGGACCTCCAGCAGTGTCTGTTGACCCACGAGCAACGCGCGCAGGACCGA

301 AlaLeuAlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArgValValLeu  
GCTTTGGCCGCGTATGCTGTCAACAGGCTGCGTGGTCAATAGTGGCAGGGTCTGCTTG  
CGAAACCGCGCATACGGACAGTTGTCCGACGCACCAGTATCACCGTCCCGCAGAAC

-----Overlap with 81-----

361 SerGlyLysProAlaIleIleProAspArgGluValLeuTyrArg  
TCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTCCCTTACCGAG  
AGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTC

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FIG. 6 Combined ORF of DNAs 36 &amp; 81

1 AspAlaHisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAla  
 GATGCCCACTTTCTATCCAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCG  
 CTACGGGTGAAAGATAGGGTCTGTTTCGTCTCACCCCTCTGGAAGGAATGGACCATGCG

61 TyrGlnAlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrp  
 TACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCCTCCCCATCGTGGGACCAGATGTGG  
 ATGTTTCGGTGGCACACGCGATCCCGAGTTCGGGGAGGGGTAGCACCTGGTCTACACC

121 LysCysLeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeu  
 AAGTGTTTGATTTCGCCTCAAGCCCACCTCCATGGGCCAACACCCCTGCTATACAGACTG  
 TTCACAACTAAGCGGAGTTCGGGTGGGAGGTACCCGGTGTGGGGACGATATGTCTGAC

181 GlyAlaValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIleMetThrCys  
 GGCCTGTTCAGAAATGAAATCACCTGACGCACCCAGTCACCAAATACATCATGACATGC  
 CCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGGTCACTGGTTTATGTAGTACTGTACG

241 MetSerAlaAspLeuGluValValThrSerThrTrpValLeuValGlyGlyValLeuAla  
 ATGTCGGCCGACCTGGAGGTCTGTCAGGACACCTGGGTGCTCGTTGGGGCGTCTCTGGCT  
 TACAGCCGGTGGACCTCCAGCAGTCTCGTGGACCCAGAGCAACCCCGCAGGACCGA

301 AlaLeuAlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArgValValLeu  
 GCTTTGGCCGCGTATTGCCTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTG  
 CGAAACCGGCGCATAACGGACAGTTGTCCGACGCACCACTATCACCCGTCCAGCAGAAC

361 SerGlyLysProAlaIleIleProAspArgGluValLeuTyrArgGluPheAspGluMet  
 TCCGGGAAGCCGCAATCATACCTGACAGGGAAGTCTCTACCGAGAGTTCGATGAGATG  
 AGGCCCTTCGGCCGTTAGTATGACTGTCCCTCAGGAGATGGCTCTCAAGCTACTCTAC

421 GluGluCysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPhe  
 GAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTC  
 CTTCTCACGAGAGTCGTGAATGGCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAG

481 LysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaPro  
 AAGCAGAAGGCCCTCGGCCTCTGTCAGACCGGCTCCCGTCAGGCAGAGGTTATCGCCCCCT  
 TTCGTCTTCGGGAGCCGGAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGA

541 AlaValGlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPhe  
 GCTGTCCAGACCACTGGCAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAACCTTC  
 CGACAGGTCTGGTTGACCGTTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAG

601 IleSerGlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAla  
 ATCAGTGGGATACAATACTTGGCGGGCTTGTCACGCTGCCGTGTAACCCCGCCATTGCT  
 TAGTCACCCCTATGTTATGAACCGCCCGAACAGTTGCGACGGACCATTTGGGGCGGTAACGA

661 SerLeuMetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGln  
 TCATTGATGGCTTTTACAGCTGCTGTACACGCCCACTAACCCTAGCCAAA  
 AGTAACTACCGAAAATGTGACGACAGTGGTGGGTGATTGGTGATCGGTTT

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FIG. 7 Translation of DNA 32

-----Overlap with 81-----  
PheThrAlaAlaValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsnIleLeu  
1 CTTTACAGCTGCTGTCACCAGCCCACTAACCAGTACCCAAACCCCTCCTCTTCAACATAT  
GAAATGTCGACGACAGTGGTCGGTGATTGGTGATCGGTTTGGGAGGAGAAGTTGTATA

GlyGlyTrpValAlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheValGlyAla  
61 TGGGGGGGTGGGTGGCTGCCCAGCTCGCCGCCCCCGGTGCGCTACTGCCTTTGTGGGCG  
ACCCCCCACCACCGACGGGTCGAGCGGCGGGGCCACGGCGATGACGGAACACCCGC

GlyLeuAlaGlyAlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAspIleLeu  
121 CTGGCTTAGCTGGCGCCGCCATCGGCAGTGTGGACTGGGAAGGTCCTCATAGACATCC  
GACCGAATCGACCGCGCGGTAGCCGTCACACCTGACCCCTTCAGGAGTATCTGTAGG

AlaGlyTyrGlyAlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSerGlyGlu  
181 TTGCAGGGTATGGCGCGGGCGTGGCGGGAGCTCTTGTGGCATTCAAGATCATGAGCGGTG  
AACGTCCCATACGCGCGCCGACCGCCCTCGAGAACACCGTAAGTTCTAGTACTCGCCAC

ValProSerThrGluAspLeuValAsnLeuLeuProAlaIleLeuSerProGlyAlaLeu  
241 AGGTCCCTCCACGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCGGAGCCC  
TCCAGGGGAGGTGCCCTCCTGGACCAGTTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGG

ValValGlyValValCysAlaAlaIleLeuArgArgHisValGlyProGlyGluGlyAla  
301 TCGTAGTCGGCGTGGTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCCGGCGAGGGGG  
AGCATCAGCCGACAGACACGTCGTTATGACGCGGCGGTGCAACCGGGCCGCTCCCCC

ValGlnTrpMetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSer  
361 CAGTGCAGTGGATGAACCGGCTGATAGCCTTCGCCTCCCGGGGAACCATGTTTCCCC  
GTCACGTCACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGG

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**FIG. 8** Translation of DNA 35

1 SerIleGluThrIleThrLeuProGlnAspAlaValSerArgThrGlnArgArgGlyArg  
 TCCATTGAGACAATCACGCTCCCCCAGGATGCTGTCTCCCGCACTCAACGTGCGGGCAGG  
 AGGTAACTCTGTTAGTGCAGGGGGTCTACGACAGAGGGCGTGAGTTGCAGCCCCGTCC  
  
 61 ThrGlyArgGlyLysProGlyIleTyrArgPheValAlaProGlyGluArgProSerGly  
 ACTGGCAGGGGGAAGCCAGGCATCTACAGATTTGTGGCACGGGGGAGCGCCCCCTCCGGC  
 TGACCGTCCCCCTTCGGTCCGTAGATGTCTAAACACCGTGGCCCCCTCGCGGGGAGGCGG  
  
 121 MetPheAspSerSerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeu  
 ATGTTGCACTCGTCCGTCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGCTC  
 TACAAGCTGAGCAGGCAGGAGACACTACGATACTGCGTCCGACACGAACCATACTCGAG  
  
 181 ThrProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThrProGlyLeuProVal  
 ACGCCCGCCGAGACTACAGTTAGGCTACGAGCGTACATGAACACCCCGGGGCTTCCCGTG  
 TCGGGCGGCTCTGATGTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGCGAC  
  
 241 CysGlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeuThrHisIleAspAla  
 TGCCAGGACCATCTTGAATTTTGGGAGGGCGTCTTACAGGCCTCACTCATATAGATGCC  
 ACGGTCCTGGTAGAAGTTAAACCCCTCCCGCAGAAATGTCCGGAGTGAGTATATCTACGG  
  
 301 HisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGln  
 CACTTTCATCCAGACAAAGCAGAGTGGGGAGAACTTCCTTACCTGGTAGCGTACCAA  
 GTGAAAGATAGGGTCTGTTTCGTCTCACCCCTCTTGAAGGAATGGACCATCGCATGGTT  
  
 -----Overlap with 36-----  
 361 AlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCys  
 GCCACCGTGTGCGCTAGGGCTCAAGCCCTCCCCATCGTGGGACCAGATGTGGAAGTGT  
 CGGTGGCACACGCGATCCCGAGTTCGGGGAGGGGGTAGCACCTGGTCTACACCTTACA  
  
 421 LeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAla  
 TTGATTCGCCTCAAGCCACCCCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCT  
 AACTAAGCGGAGTTCGGGTGGGAGGTACCCGTTGTGGGGACGATATGTCTGACCCGCGA

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FIG. 9-1 Combined ORF of DNAs 35, 36, 81 &amp; 32

SerIleGluThrIleThrLeuProGlnAspAlaValSerArgThrGlnArgArgGlyArg  
 1 TCCATTGAGACAATCACGCTCCCCAGGATGCTGCTCCCGCACTCAACGTCGGGGCAGG  
 AGGTAACCTCTGTTAGTGCAGGGGGTCTACGACAGAGGGCGTGAGTTGCAGCCCGCTCC

ThrGlyArgGlyLysProGlyIleTyrArgPheValAlaProGlyGluArgProSerGly  
 61 ACTGGCAGGGGAAGCCAGGCATCTACAGATTTGTGGCACCAGGGGAGCGCCCTCCGGC  
 TGACCGTCCCCCTTCGGTCCGTAGATGTCTAACACCGTGGCCCCCTCGCGGGGAGGCCG

MetPheAspSerSerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeu  
 121 ATGTTCCGACTCGTCCGTCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGCTC  
 TACAAGCTGAGCAGGCAGGAGACACTCACGATACTGCGTCCGACACGAACCATACTCGAG

ThrProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThrProGlyLeuProVal  
 181 ACGCCCGCCGAGACTACAGTTAGGCTACGAGCGTACATGAACACCCCGGGGCTTCCCGTG  
 TCGGGGCGGCTCTGATGTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGGCAC

CysGlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeuThrHisIleAspAla  
 241 TGCCAGGACCATCTTGAATTTGGGAGGGCGTCTTACAGGCCCTCACTCATATAGATGCC  
 ACGGTCCTGGTAGAACTTAAACCCCTCCCGCAGAAATGTCCGGAGTGAGTATATCTACGG

HisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGln  
 301 CACTTTCTATCCAGACAAAGCAGAGTGGGGAGAACCTTCCCTACCTGGTAGCGTACCAA  
 GTGAAAGATAGGGTCTGTTTCTGCTCACCCCTCTTGAAGGAATGGACCATCGCATGGTT

AlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCys  
 361 GCCACCGTGTGCGCTAGGGCTCAAGCCCTCCCCATCGTGGGACCAGATGTGGAAGTGT  
 CGGTGGCACACGCGATCCCGAGTTCGGGGAGGGGGTAGCACCTGGTCTACACCTTCACA

LeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAla  
 421 TTGATTGCGCTCAAGCCACCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCT  
 AACTAAGCGGAGTTCGGGTGGGAGGTACCCGGTTGTGGGGACGATATGTCTGACCCGCA

ValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIleMetThrCysMetSer  
 481 GTTCAGAATGAAATCACCTGACGCACCCAGTCACCAAATACATCATGACATGCATGTCTG  
 CAAGTCTTACTTTAGTGGGACTGCGTGGGTGAGTGGTTATGTAGTACTGTACGTACAGC

AlaAspLeuGluValValThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeu  
 541 GCCGACCTGGAGGTGCTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCTGGCTGCTTTG  
 CGGTGGACCTCCAGCAGTGTCTGTTGACCCACGAGCAACGCGCGCAGGACCGACGAAAC

AlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArgValValLeuSerGly  
 601 GCCGCGTATTGCTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTGTCCGGG  
 CGGCGCATAACGGACAGTTGTCCGAAGCACCAGTATCACCGTCCCGCAGAACAGGCC

LysProAlaIleIleProAspArgGluValLeuTyrArgGluPheAspGluMetGluGlu  
 661 AAGCCCGCAATCATACCTGACAGGGAAGTCTCTACCGAGAGTTCGATGAGATGGAAGAG  
 TTCGGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTCTC

CysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPheLysGln  
 721 TGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAG  
 ACGAGAGTCTGTAATGGCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCTGTC

LysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaProAlaVal  
 781 AAGGCCCTCGGCCCTCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCCTGCTGTC  
 TTCCGGGAGCCGGAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGACGACAG

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841 GlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPheIleSer  
 CAGACCAACTGGCAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAAC TTCATCAGT  
 GTCTGGTTGACCGTTTTTGTAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAGTAGTCA

901 GlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAlaSerLeu  
 GGGATACAATACTTGGCGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATGT  
 CCCTATGTTATGAACCGCCGAACAGTTGCGACGGACCATGGGGCGGTAACGAAGTAAC

961 MetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsn  
 ATGGCTTTTACAGCTGCTGTCAACGACCCACTAACCCTAGCCAAACCTCCTCTTCAAC  
 TACCGAAAATGTCGACGACAGTGGTCGGGTGATTGGTGATCGGTTTGGGAGGACAAGTTG

1021 IleLeuGlyGlyTrpValAlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheVal  
 ATATTGGGGGGTGGGTGGCTGCCAGCTCGCCGCCCGGTCGCGCTACTGCCTTTGTG  
 TATAACCCCCCACCACCGACGGGTCGAGCGGCGGGGCCAGGCGATGACGGAAACAC

1081 GlyAlaGlyLeuAlaGlyAlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAsp  
 GGCGCTGGCTTAGCTGGCGCCGCCATCGGCAGTGTGGACTGGGGAAGGTCTTCATAGAC  
 CCGCGACCGAATCGACCGCGCGGTAGCCGTACAACTGACCCCTTCCAGGAGTATCTG

1141 IleLeuAlaGlyTyrGlyAlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSer  
 ATCCTTGCGAGGTATGGCGCGGGCGTGGCGGGAGCTCTTGTGGCATTCAGATCATGAGC  
 TAGGAACGTCCCATACCGCGCCCGCACCGCCCTCGAGAACACCGTAAGTTCTAGTACTCG

1201 GlyGluValProSerThrGluAspLeuValAsnLeuLeuProAlaIleLeuSerProGly  
 GGTGAGGTCCCTCCACGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCGGA  
 CCACTCCAGGGGAGGTGCTCTGACCAAGTTAGATGACGGCGGTAGGAGAGCGGGCCT

1261 AlaLeuValValGlyValValCysAlaAlaIleLeuArgArgHisValGlyProGlyGlu  
 GCCCTCGTAGTCGGCGTGGTCTGTGCAGCAATACTGCGCCGCGCACGTTGGCCCGGGCGAG  
 CGGGAGCATCAGCCGCACACAGACACGTCGTTATGACGCGGCGGTGCAACCGGGCCCGCTC

1321 GlyAlaValGlnTrpMetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSer  
 GGGGCAGTGCAGTGGATGAACCGGCTGATAGCCTTCGCCTCCGGGGGAACCATGTTTCCCC  
 CCGCGTCACGTCACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGG

FIG. 9-2

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## FIG. 10

Translation of DNA 37b

1 LeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGlyLeuAsp  
 CTCGCCGCAAAGCTGGTCGCATTGGGCATCAATGCCGTGGCCTACTACCGCGGTCTTGAC  
 GAGCGCGCTTTCGACCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCAGAAGT  
  
 61 ValSerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeuMetThr  
 GTGTCCGTCATCCCGACCGAGCGGCGATGTTGTCGTCGTGGCAACCGATGCCCTCATGACC  
 CACAGGCAGTAGGGCTGGTCGCCGCTACAACAGCAGCACCGTTGGCTACGGGAGTACTGG  
  
 121 GlyTyrThrGlyAspPheAspSerValIleAspTyrAsnThrCysValThrGlnThrVal  
 GGCTATACCGGCGACTTCGACTCGGTGATAGACTACAATACGTGTGTCACCCAGACAGTC  
 CCGATATGGCCGCTGAAGCTGAGCCACTATCTGATGTTATGCACACAGTGGGTCTGTACG  
  
 -----Overlap with  
 181 AspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAspAlaVal  
 GATTTTCAGCCTTGACCTTACCTTCACCATGAGACAATCACGCTCCCCCAGGATGCTGTC  
 CTAAGTCGGAAGTGGGATGGAAGTGGTAACTCTGTTAGTGGAGGGGGTCTACGACAG  
  
 clone 35-----  
 241 SerArgThrGlnArgArgGlyArgThr  
 TCCCGCACTCAACGTCGGGGCAGGACTG  
 AGGGCGTGAGTTGCAGCCCCGTCCTGAC

## FIG. 11

Translation of DNA 33b

-----Overlap with 32-----  
 1 MetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrVal  
 GATGAACCGGCTGATAGCCTTCGCCTCCCGGGGAACCATGTTTCCCCACGCACTACGT  
 CTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGGTGCCTGATGCA  
  
 61 ProGluSerAspAlaAlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGln  
 GCCGGAGAGCGATGCAGCTGCCCGCTCACTGCCATACTCAGCAGCTCACTGTAACCCA  
 CGGCCTCTCGCTACGTCGACGGGCGCAGTGACGGTATGAGTCGTGGAGTGACATTGGGT  
  
 121 LeuLeuArgArgLeuHisGlnTrpIleSerSerGluCysThrThrProCysSerGlySer  
 GCTCCTGAGGCGACTGCACCACTGGATAAGCTCGGAGTGTACCACTCCATGCTCCGGTTC  
 CGAGGACTCCGCTGACGTGGTCACCTATTCGAGCCTCATATGGTGAGGTACGAGGCCAAG  
  
 181 TrpLeuArgAspIleTrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeu  
 CTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGTGAGCGACTTTAAGACCTGGCT  
 GACCGATTCCCTGTAGACCTGACCTATACGCTCCACAACCTCGCTGAAATCTGGACCGA  
  
 241 LysAlaLysLeuMetProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyr  
 AAAAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCTTTGTGTCTGCCAGCGCGGTA  
 TTTTCGATTTCGAGTACGGTGTGCACGGACCTAGGGGAAACACAGGACGGTCGCGCCCAT  
  
 301 LysGlyValTrpArgVal  
 TAAGGGGGTCTGGCGAGTG  
 ATTCCCCCAGACCGCTCAC

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FIG. 12 Translation of DNA 40b

AlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThrGlyValArgThrIle  
 1 GGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACCGGGGTGAGAACAAAT  
 CCGAATGTACAGGTTCCGAGTACCCTAGCTAGGATTGTAGTCTGGCCCCACTCTTGTTA

ThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCys  
 61 TACCACTGGCAGCCCCATCACGTACTCCACCTACGGCAAGTTCCTTGCCGACGGCGGGTG  
 ATGGTGACCGTCGGGGTAGTGATGAGGTGATGCCGTTCAAGGAACGGCTGCCGCCAC

SerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSerThrAspAlaThrSer  
 121 CTCGGGGGGCGCTTATGACATAATAATTTGTGACGAGTGCCACTCCACGGATGCCACATC  
 GAGCCCCCGCGAATACTGTATTATTAAACTGCTCACGGTGAGGTGCCCTACGGTGTAG

IleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValVal  
 181 CATCTTGGGCATCGGCATGTCCTTGACCAAGCAGAGACTGCGGGGGCGAGACTGGTTGT  
 GTAGAACCCGTAGCCGTGACAGGAAGTGGTTCGTCTCTGACGCCCCCGCTTGACCAACA

LeuAlaThrAlaThrProProGlySerValThrValProHisProAsnIleGluGluVal  
 241 GCTCGCCACCGCCACCCCTCCGGGCTCCGTCACTGTGCCCCATCCCAACATCGAGGAGGT  
 CGAGCGGTGGCGGTGGGGAGGCCGAGGCAGTGACACGGGGTAGGGTTGTAGCTCTCCA

AlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIleProLeuGluValIle  
 301 TGCTCTGTCCACCACCGAGAGATCCCTTTTACGGCAAGGCTATCCCCCTCGAAGTAAT  
 ACGAGACAGGTGGTGGCTCTCTAGGGAAAAATGCCGTTCCGATAGGGGGAGCTTCATTA

-----  
 LysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCysAspGluLeuAlaAla  
 361 CAAGGGGGGAGACATCTCATCTTCTGTCAATCAAAGAAGAAGTGCGACGAACTCGCCGC  
 GTTCCCCCTCTGTAGAGTAGAAGACAGTAAGTTTCTTCTTCACGCTGCTTGAGCGGCG

-----Overlap with 37b-----  
 LysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerVal  
 421 AAAGCTGGTCGCAATTGGGCATCAATGCCGTGGCCTACTACCGCGGTCTTGACGTGTCCGT  
 TTTCGACCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCAGAAGTGCACAGGCA

-----  
 IleProThr  
 481 CATCCCGACCAG  
 GTAGGGCTGGTC

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FIG. 13 Translation of DNA 25c

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-----
1  CysSerLeuThrValThrGlnLeuLeuArgArgLeuHisGlnTrpIleSerSerGluCys
   ACTGCAGCCTCACTGTAACCCAGCTCCTGAGGCGACTGCACCAGTGGATAAGCTCGGAGT
   TGACGTGCGGAGTGACATTGGGTGAGGACTCCGCTGACGTGGTCACCTATTTCGAGCCTCA
-----
61  ThrThrProCysSerGlySerTrpLeuArgAspIleTrpAspTrpIleCysGluValLeu
   GTACCACTCCATGCTCCGGTTCCTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGT
   CATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAGACCTGACCTATACGCTCCACA
-----Overlap with 33b-----
121 SerAspPheLysThrTrpLeuLysAlaLysLeuMetProGlnLeuProGlyIleProPhe
   TGAGCGACTTTAAGACCTGGCTAAAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCTT
   ACTCGCTGAAATCTGGACCGATTTTCGATTTCGAGTACGGTGTGACGGACCTTAGGGGA
-----
181 ValSerCysGlnArgGlyTyrLysGlyValTrpArgGlyAspGlyIleMethisThrArg
   TTGTGCTCTGCCAGCGCGGTATAAGGGGTCTGGCGAGGGGACGGCATCATGCACACTC
   AACACAGGACGGTCGCGCCCATATTCCCCAGACCGCTCCCTGCCGTAGTACGTGTGAG
-----
241 CysHisCysGlyAlaGluIleThrGlyHisValLysAsnGlyThrMetArgIleValGly
   GCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAAAACGGGACGATGAGGATCGTCTG
   CGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTTTTGCCCTGCTACTCCTAGCAGC
-----
301 ProArgThrCysArgAsnMetTrpSerGlyThrPheProIleAsnAlaTyrThrThrGly
   GTCCTAGGACCTGCAGGAACATGTGGAGTGGGACCTTCCCCATTAATGCCTACACCACGG
   CAGGATCCTGGACGTCCTTGTACACCTCACCTGGAAGGGTAATTACGGATGTGGTGCC
-----
361 ProCysThrProLeuProAlaProAsnTyrThrPheAlaLeuTrpArgValSerAlaGlu
   GCCCCGTGACCCCCCTTCTGCGCCGAACACACGTTCCGCGCTATGGAGGGTGTCTGCAG
   CGGGGACATGGGGGAAGGACGCGCTTGATGTGCAAGCGGATACCTCCACAGACGTC
-----
421 GluTyrValGluIleArgGlnValGlyAspPheHisTyrValThrGlyMetThrThrAsp
   AGGAATATGTGGAGATAAGGCAGGTGGGGGACTTCCACTACGTGACGGGTATGACTACTG
   TCCTTATACACCTCTATTCCGTCCACCCCTGAAGGTGATGCACTGCCATACTGATGAC
-----
481 AsnLeuLysCysProCysGlnValProSerProGluPhePheThrGlu
   ACAATCTCAAATGCCGTGCCAGGTCCCATCGCCGAATTTTCACAGAAT
   TGTTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTTAAAAAGTGTCTTA

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FIG. 14-1 Combined ORF of DNAs 40b/37b/35/36/81/32/33b/25c

1 AlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThrGlyValArgThrIle  
 TGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACCGGGGTGAGAACAAT  
 ACGAATGTACAGGTTCCGAGTACCCTAGCTAGGATTGTAGTCTGGCCCCACTCTTGTTA  
 61 ThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCys  
 TACCACTGGCAGCCCCATCAGTACTCCACCTACGGCAAGTTCCTTGCCGACGGCGGGTG  
 ATGGTGACCGTCGGGGTAGTGCATGAGGTGGATGCCGTTCAAGGAACGGCTGCCGCCCCAC  
 121 SerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSerThrAspAlaThrSer  
 CTCGGGGGGCGCTTATGACATAATAATTGTGACGAGTGCCACTCCACGGATGCCACATC  
 GAGCCCCCGCGAATACTGTATTATTAAACACTGCTCACGGTGAGGTGCCCTACGGTGATG  
 181 IleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValVal  
 CATCTTGGGCATCGGCACTGTCTTGACCAAGCAGAGACTGCGGGGGCAGACTGGTTGT  
 GTAGAACCCGTAGCCGTGACAGGAACGTGGTTCGTCTCTGACGCCCCCGCTCTGACCAACA  
 241 LeuAlaThrAlaThrProGlySerValThrValProHisProAsnIleGluGluVal  
 GCTCGCCACCGCCACCCCTCCGGGCTCCGTCACTGTGCCCATCCCAACATCGAGGAGGT  
 CGAGCGGTGGCGGTGGGGAGGCCCGAGGCAGTGACACGGGTAGGGTTGTAGCTCCTCCA  
 301 AlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIleProLeuGluValIle  
 TGCTCTGTCCACCACCGAGAGATCCCTTTTACGGCAAGGCTATCCCCCTCGAAGTAAT  
 ACGAGACAGGTGGTGGCCTCTCTAGGGAAAAATGCCGTTCCGATAGGGGGAGCTTCATTA  
 361 LysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCysAspGluLeuAlaAla  
 CAAGGGGGGAGACATCTCATTTCTGTCAATCAAAGAAGAAGTGCGACGAACCTCGCCGC  
 GTTCCCCCTCTGTAGAGTAGAAGACAGTAAGTTTCTTCTTACGCTGCTTGAGCGGGC  
 421 LysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerVal  
 AAAGCTGGTTCGCTTGGGCATCAATGCCGTGGCCTACTACCGGGTCTTGACGTGTCCGT  
 TTTCCAGCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCAGAACTGCACAGGCA  
 481 IleProThrSerGlyAspValValValAlaThrAspAlaLeuMetThrGlyTyrThr  
 CATCCCGACCGCGCGATGTTGTCTGTGGCAACOGATGCCCTCATGACCGGCTATAC  
 GTAGGGCTGGTCGCGCTACAAACAGCAGCACCGTTGGCTACGGGAGTACTGGCCGATAG  
 541 GlyAspPheAspSerValIleAspTyrAsnThrCysValThrGlnThrValAspPheSer  
 CGGCGACTTCGACTCGGTGATAGACTACAATACGTGTGTACCCAGACAGTCCGATTTCAG  
 GCCGCTGAAGCTGAGCCACTATCTGATGTTATGCACACAGTGGGTCTGTACGTAAAGTC  
 601 LeuAspProThrPheThrIleGluThrIleThrLeuProGlnAspAlaValSerArgThr  
 CCTTGACCCCTACCTTCCACATTGAGACAATCACGCTCCCCAGGATGCTGTCTCCCGCAC  
 GGAAGTGGGATGGAAGTGGTAACCTCTGTAGTGGCAGGGGGTCTACGACAGAGGGCGTG  
 661 GlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArgPheValAlaProGly  
 TCAACGTCGGGGCAGGACTGGCAGGGGAAGCCAGGCATCTACAGATTGTGGCACCGGG  
 AGTTGCAGCCCCCTCCTGACCGTCCCTTCGGTCCGTAGATGTCTAAACACCGTGGCCC  
 721 GluArgProSerGlyMetPheAspSerSerValLeuCysGluCysTyrAspAlaGlyCys  
 GGAGCGCCCCCTCCGGCATGTTGACTCGTCCGTCTCTGTGAGTGCTATGACGAGGCTG  
 CCTCGCGGGGAGGCGGTACAAGCTGAGCAGGAGGAGACTCAGGATACTGCGTCCGAC  
 781 AlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThr  
 TGCTTGGTATGAGCTACGCCCCGAGACTACAGTTAGGCTACGAGCGTACATGAACAC  
 ACGAACCATACTGAGTGGGGCGGCTCTGATGTCAATCCGATGCTCGCATGTACTTGTG  
 841 ProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeu  
 CCGGGGGCTTCCGTGTGCGAGGACCATCTGAATTTGGGAGGGCGCTTTACAGGCCCT  
 GGGCCCCGAAGGGCACACGGTCTGGTAGAAGTTAAACCCCTCCCGCAGAAATGTCCGGA  
 901 ThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyr  
 CACTATATAGATGCCACTTTCTATCCAGACAAAGCAGAGTGGGAGAACCTTCCTTA  
 GTGAGTATATCTACGGGTGAAAGATAGGGTCTGTTTCTCTACCCCTCTTGGGAAGGAAT

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961 LeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaProProSerTrpAsp  
 CCTGGTAGCGTAOCCAAGCCACCGTGTGGCTAGGGCTCAAGCCCTCCOCCATCGTGGGA  
 GGACCATCGCATGGTTCCGTGGCACACGCGATCCGAGTTCGGGGAGGGGGTAGCACCTT  
 1021 GlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeu  
 CCAGATGTGAAGTGTGTTGATTGCGCTCAAGCCACCCCTCCATGGGCCAACACCCCTGCT  
 GGTCTACACCTTCACAACTAAGCGGAGTTCGGGTGGGAGGTACCCGGTTGTGGGGACGA  
 1081 TyrArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIle  
 ATACAGACTGGGCGCTGTTTCAAGATGAAATCACCTGACGCACCCAGTACCAAATACAT  
 TATGTCGTACCCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGGTGAGTGGTTTATGTA  
 1141 MetThrCysMetSerAlaAspLeuGluValValThrSerThrTrpValLeuValGlyGly  
 CATGACATGTCATGTCCGCCGACCTGGAGGTGCTCACGAGCACCTGGGTGCTGTTGGCGG  
 GTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTGTCTGTGGACCCACGAGCAACCGCC  
 1201 ValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArg  
 CGTCTGGCTGCTTTGGCGCGTATGCTGTCAACAGGCTGCGTGGTCATAGTGGGCAG  
 GCAGGACCGACGAAACCGGCGCATAACGGACAGTTGTCCGACGCCACAGTATCACCCGTC  
 1261 ValValLeuSerGlyLysProAlaIleIleProAspArgGluValLeuTyrArgGluPhe  
 GGTGCTCTTGTCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTCTCTTACCGAGAGTT  
 CCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGACTGTCCTTCAGGAGATGGCTCTCAA  
 1321 AspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAla  
 CGATGAGATGGAAGAGTGTCTCAGCACTTACCGTACATCGAGCAAGGGAATGATGTCCG  
 GCTACTCTACCTTCTCAGAGAGTGTGAATGGCATGTAGCTGTTCCCTACTACGAGCG  
 1381 GluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluVal  
 CGAGCAGTTCAAGCAGAAGGCCCTCGGCCCTCTGCAGACCCGCTCCCGTCAGGCAGAGGT  
 GCTCGTCAAGTTCGTTCTCCGGGAGCCGGAGGACGCTCTGGCGCAGGCAGTCCGCTCTCA  
 1441 IleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMet  
 TATCGCCCTGCTGTCCAGACCACTGGCAAACTCGAGACCTTCTGGGCGAAGCATAT  
 ATAGCGGGACGACAGGTCTGGTTGACCGTTTTTGGAGCTCTGGAAGACCGCTTCGTATA  
 1501 TrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnPro  
 GTGGAACATCATCAGTGGGATACAATACTTGGCGGGCTGTGCAACGCTGCTGGTAACCC  
 CACCTTGAAGTAGTCACCTATGTTATGAACCGCCGAAACAGTTGCGACGGACCATTGGG  
 1561 AlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGln  
 CGCCATTGCTTCATTGATGGCTTTTACAGCTGCTGTACACAGCCCACTAACCCTAGCCA  
 GCGGTAAAGTAAGTAACGAAATGTGACGACAGTGGTGGGTGATTGGTGATCGGT  
 1621 ThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeuAlaAlaProGlyAla  
 AACCTCTCTTCAACATATTGGGGGGTGGGTGGCTGCCAGCTCGCCGCCCGCGGTGC  
 TTGGGAGGAGAAGTTGTATAACCCCCCACCACCGACGGGTGAGCGGGCGGGGCCACG  
 1681 AlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGlySerValGlyLeuGly  
 CGCTACTGCTTGTGGGGCGTGGCTTAGCTGGGCGCGCCATCGGCAGTGTGGACTGGG  
 GCGATGACGAAACACCGCGACGGAATCGACCGCGGGTAGCGCTACAACTGACCC  
 1741 LysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAlaGlyAlaLeuValAla  
 GAAGGTCTCATAGACATCTTGCAGGGTATGGGCGGGCGTGGCGGGAGCTCTTGTGGC  
 CTTCCAGGAGTATCTGTAGGAACGTCCCATACGCGCGCGCACCGCCCTGAGAACACCG  
 1801 PheLysIleMetSerGlyGluValProSerThrGluAspLeuValAsnLeuLeuProAla  
 ATTCAAGATCATGAGCGGTGAGGTCCCTCCACGAGGACCTGGTCAATCTACTGCCCGC  
 TAAGTTCTAGTACTCGCCACTCCAGGGGAGGTGCTCTGACAGTGTAGATGACGGCG  
 1861 IleLeuSerProGlyAlaLeuValValGlyValValCysAlaAlaIleLeuArgArgHis  
 CATCCTCTCGCCCGGAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCAATACTGCGCGGCA  
 GTAGGAGAGCGGGCTCGGGAGCATCAGCCGACACGACAGTGGTTATGAGCGCGCT

FIG. 14-2 valGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIleAlaPheAlaSerArg

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1921 CGTTGGCCCCGGCGAGGGGCGAGTGCAGTGGATGAACCGGCTGATAGCCTTCGCCCTCCCC  
 GCAACCGGGCCCGCTCCCCCGTCAGTCACCTACTTGGCCGACTATCGGAAGCGGAGGGC  
  
 GlyAsnHisValSerProThrHisTyrValProGluSerAspAlaAlaAlaArgValThr  
 1981 GGGGAACCATGTTTCCCCCAGCACTACGTGCCGGAGAGCGATGCAGCTGCCCGCGTCAC  
 CCCCTTGGTACAAAGGGGTGCGTGATGCACGCCCTCTCGTACGTGACGGGCGCAGTG  
  
 AlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeuHisGlnTrpIleSer  
 2041 TGCCATACTCAGCAGCCTCACTGTAACCCAGCTCCTGAGGCGACTGCACCAAGTGATAAG  
 ACGGTATGAGTCGTGCGAGTGACATTGGGTGAGGACTCCGCTGACGTGGTCACCTATTC  
  
 SerGluCysThrThrProCysSerGlySerTrpLeuArgAspIleTrpAspTrpIleCys  
 2101 CTCGGAGTGTAACCACTCCATGCTCCGGTTCCTGGCTAAGGACATCTGGGACTGGATATG  
 GAGCTCACATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAGACCTGACCTATAC  
  
 GluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMetProGlnLeuProGly  
 2161 CGAGGTGTTGAGCGACTTTAAGACCTGGCTAAAAGCTAAGCTCATGCCACAGCTGCCTGG  
 GCTCCACAACCTCGCTGAAATTCTGGACCGATTTTCGATTGAGTACGGTGTGACGGACC  
  
 IleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArgValAspGlyIleMet  
 2221 GATCCCCCTTTGTGTCTGCCAGCGCGGGTATAAGGGGGTCTGGCGAGTGGACGGCATCAT  
 CTAGGGGAAACACAGGACGGTCGCGCCATATTCCCCAGACCGCTCACCTGCCGTAGTA  
  
 HisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLysAsnGlyThrMetArg  
 2281 GCACACTCGCTGCCACTGTGGAGCTGAGATCAGTGGACATGTCAAAAACGGGACGATGAG  
 CGTGTGAGCGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTTGTCCCTGCTACTC  
  
 IleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPheProIleAsnAlaTyr  
 2341 GATCGTCGGTCCTAGGACCTGCAGGAACATGTGGAGTGGGACCTTCCCCATTAATGCCTA  
 CTAGCAGCCAGGATCCTGGACGTCTTGTACACCTCACCTGGAAGGGGTAATTACGGAT  
  
 ThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPheAlaLeuTrpArgVal  
 2401 CACCACGGGCCCCCTGTACCCCCCTTCTGCGCGAACTACACGTTGCGCTATGGAGGGT  
 GTGGTGCCCCGGGACATGGGGGAAGGACGCGGCTTGATGTGCAAGCGGATACCTCCCA  
  
 SerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHisTyrValThrGlyMet  
 2461 GTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGACTTCCACTACGTGACGGGTAT  
 CAGACGTCTCCTTATACACCTTATTCCGTCCACCCCTGAAGGTGATGCACTGCCATA  
  
 ThrThrAspAsnLeuLysCysProCysGlnValProSerProGluPhePheThrGlu  
 2521 GACTACTGACAATCTCAAATGCCCGTGCCAGGTCCCATCGCCCGAATTTTTCACAGAAT  
 CTGATGACTGTTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTAAAAAGTGCTCTTA

FIG. 14-3

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FIG. 15 Translation of DNA 33c

AlaValAspPheIleProValGluAsnLeuGluThrThrMetArgSerProValPheThr  
 1 GCGCGTGGACTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCGGTGTTTCAC  
 CCGCCACCTGAAATAGGGACACCTCTTGGATCTCTGTGGTACTCCAGGGGCCACAAGTG

AspAsnSerSerProProValValProGlnSerPheGlnValAlaHisLeuHisAlaPro  
 61 GGATAACTCCTCTCCACAGTAGTGTCCCGAGAGCTTCCAGGTGGCTCACCTCCATGCTCC  
 CCTATTGAGGAGAGGTGGTCATCACGGGTCTCGAAGGTCCACCGAGTGGAGGTACGAGG

ThrGlySerGlyLysSerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLysVal  
 121 CACAGGCGAGCGGCAAAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATAAGGT  
 GTGTCCGTGCGCGTTTCTGTGGTTCCAGGGCCGACGTATACGTGAGTCCCGATATTCCA

LeuValLeuAsnProSerValAlaAlaThrLeuGlyPheGlyAlaTyrMetSerLysAla  
 181 GCTAGTACTCAACCCCTCTGTGTGCTGCAACACTGGGCTTGGGTGCTTACATGTCCAGGC  
 CGATCATGAGTTGGGGAGACAACGACGTTGTGACCGAAACCAGAAATGTACAGGTTCGG

-----Overlap with 40b-----  
 HisGlyIleAspProAsnIleArgThrGlyValArgThrIleThrThrGlySerProIle  
 241 TCATGGGATCGATCCTAACATCAGGACCGGGGTGAGAACAAATTACCACTGGCAGCCCCAT  
 AGTACCCTAGCTAGGATTGTAGTCTGCGCCCACTCTTGTAAATGGTGACCGTGGGGTA

ThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyrAsp  
 301 CACGTACTCCACCTACCGCAAGTTCCTTCCCGACGGCGGGTGCTCGGGGGGCGCTTATGA  
 GTGCATGAGGTGGATGCGGTTCAGGAACGGCTGCCGCCACGAGCCCCCGCGAATACT

IleIleIleCysAspGluCysHisSerThrAspAlaThrSerIleLeuGlyIleGlyThr  
 361 CATAATAATTGTGACGAGTGCCACTCCAGGATGCCACATCCATCTTGGGCATTTGGCAC  
 GTATTATTAAACACTGTCTACGGTGAGGTGCCTACGGTGTAGGTAGAACCCGTACCGTG

ValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrPro  
 421 TGTCTCTGACCAAGCAGAGACTGCGGGGCGGAGACTGGTTGTGCTCGCCACCGCCACCCC  
 ACAGGAACCTGGTTCGTCTCTGACGCCCCGCTCTGACCAACACGAGCGGTGGCGGTGGGG

ProGlySerValThrValProHisProAsnIleGluGluValAlaLeuSerThrThrGly  
 481 TCCGGGCTCCGTCACTGTGCCCCATCCCAACATCGAGGAGGTGTCTGTCTCCACCAACGG  
 AGGCCCCAGGCAGTGACAGGGGTAGGGTTGTAGCTCTCCAACGAGACAGGTGGTGGCC

GluIleProPheTyrGlyLysAlaIleProLeuGluValIleLysGlyGlyArgHisLeu  
 541 AGAGATCCCTTTTACGGCAAGGCTATCCCCCTCGAAGTAATCAAGGGGGGAGACATCT  
 TCTCTAGGGAATAATGCGGTTCCGATAGGGGGAGCTTCATTAGTTCCCCCCTCTGTAGA

IlePheCysHisSerLysLysLysCysAspGluLeuAlaAlaLysLeuValAlaLeuGly  
 601 CATCTTCTGTCTTCAAGAAGAGTGGACGAACTGCGCGAAAGCTGGTTCGATTCGGG  
 GTAGAAGACAGTAAGTTTCTTCTTACGCTGCTTGAGGGCGTTTCGACACGCGTAACCC

IleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerValIleProThrSerGlyAsp  
 661 CATCAATGCCGTGGCTACTACCGGCTCTTGACGTGTCCGTATCCGACCGAGCGGCGA  
 GTAGTTACGGCACCGGATGATGGCGCCAGAACTGCACAGGCAGTAGGGCTGGTTCGCGCT

ValValValValAlaThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSerVal  
 721 TGTGTCTGTGTGGCAACGATGCCCTCATGACGGCTATACGGCGGACTTCGACTCGGT  
 ACAACAGCAGCACCCTTGGCTACGGGAGTACTGGCCGATATGGCCGCTGAAGCTGAGCCA

IleAspCysAsnThrCys  
 781 GATAGACTGCAATACGTGTG  
 CTATCTGACGTTATGCACAC

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FIG. 16 Translation of DNA 8h

ProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIlePro  
 1 CTCCCTGCACTTGGCGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCATT  
 GAGGGACGTGAACGCGAGGAGCTGGAAATGGACCAGTGCTCGTGCGGCTACAGTAAG

ValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyrLeu  
 61 CCGTGCGCGCGCGGGTGATAGCAGGGGAGCCTGCTGTCGCCCGCGGGCAGCGGTGGCATATTTA  
 GGCACGCGGCGCGCCCACTATCGTCCCGTGGACGACAGCGGGGCGGGTAAAGGATGA

LysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePheArg  
 121 TGAAGGCTCCTCGGGGGTCCGCTGTGTCGCCCGCGGGGACGCGGTGGCATATTTA  
 ACTTTCGAGGAGCCCCCAGGCGACAACAGGGGCGCCCGTGCGGCACCCGTATAAAT

AlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsnLeu  
 181 GGGCGCGGTGTGCAACCGTGGAGTGGCTAAGGCGGTGGACTTTATCCTGTGGAGAACC  
 CCGCGCGCACACGTGGGCACCTCACGATTCCGCCACCTGAAATAGGGACACCTCTTG

33c-----Overlap with  
 GluThrThrMetArgSerProValPheThrAspAsnSer  
 241 TAGAGACACCATGAGGTCCCGGTGTTCCGGATAACTCCTC  
 ATCTCTGTTGGTACTCCAGGGGCCACAAGTGCCTATTGAGGAG

FIG. 17 Translation of DNA 7e

GlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeuGly  
 1 GGGGTGGAGGTGTGCTGGCGCCATCACGGGTACGCCAGCAGACAGGGGCTCCTAGG  
 CCCACCTCCAACGACCGCGGTAGTGCCGCATGCGGGTCTGTGTTCCCGGAGGATCC

CysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGlnIle  
 61 GTGCATAATCACCAGCCTAATGCGCGGGACAAAACCAAGTGGAGGGTGAGGTCCAGAT  
 CACGTATTAGTGGTGGATTGACCGGCCCTGTTTTGGTTCACTCCACTCCAGGTCTA

ValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThrVal  
 121 TGTGTCAACTGCTGCCAAACCTTCTGGCAACGTGCATCAATGGGGTGTGCTGGACTGT  
 ACACAGTTGACGACGGGTTTGAAGGACCGTTGCAGTAGTTACCCACACGACCTGACA

TyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMetTyr  
 181 CTACCAGGGGCGGAACGAGGACCATCGCGTCACCAAGGGTCTGTATCCAGATGTA  
 GATGTTGCCCGGCCCTGCTCCTGGTAGCGAGTGGTTCCAGGACAGTAGGTCTACAT

ThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeuThr  
 241 TACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTCCGCAGGTAGCCGCTCATTGAC  
 ATGGTTACATCTGGTTCTGGAACACCGACCGGGGAGGGGTTCCATGGCGAGTAACG

-----Overlap with 8h-----  
 ProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHis  
 301 ACCCTGCACTTGGCGCTCCTCGGACCTTTACCTGGTCACGAGGCACG  
 TGGGACGTGAACGCGAGGAGCTGGAAATGGACCAGTGCTCGTG

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FIG. 18 Translation of DNA 14c

AsnMetTrpSerGlyThrPheProIleAsnAlaTyrThrThrGlyProCysThrProLeu  
 1 GPACATGTGGAGTGGGACCTTCCCCATTAATGCCTACACCACGGGCCCCGTACCCCCCT  
 CTGTACACCTCACCTGGAAGGGTAATTACGGATGTGGTCCCCGGGGACATGGGGGA

---

Overlap with 25c

ProAlaProAsnTyrThrPheAlaLeuTrpArgValSerAlaGluGluTyrValGluIle  
 61 TCCTGCGCCGAACTACACGTTCGCGCTATGGAGGGTGTCTGCAGAGGAATACGTGGAGAT  
 AGGACGCGGCTTGATGTGCAAGCGGATACCTCCACAGAOGTCTCCTATGCACCTCA

---

ArgGlnValGlyAspPheHisTyrValThrGlyMetThrThrAspAsnLeuLysCysPro  
 121 AAGGCAGGTGGGGACTTCCACTACGTGACGGGTATGACTACTGACARTCTTAAATGCC  
 TTCCGTCCACCCCTGAAGGTGATGACTGCCATACTGATGACTGTTAGAATTTACGGG

---

CysGlnValProSerProGluPhePheThrGluLeuAspGlyValArgLeuHisArgPhe  
 181 GTGCCAGGTCCCATCGCCGAATTTTTCACAGAAATGGACGGGGTGGCCTACATAGGTT  
 CACGGTCCAGGTAGCGGGCTTAAAAAGTGTCTTAACTGCCCCACGGGATGTATCAA

---

AlaProProCysLysProLeuLeuArgGluGluValSerPheArgValGlyLeuHisGlu  
 241 TCGCCCCCCTGCAAGCCCTTGCTGCGGGAGGAGGTATCATTAGAGTAGGACTCCACGA  
 ACGCGGGGGGACGTTCCGGAAACGACGCCCTCCTCCATAGTAAGTCTCATCTGAGGTGCT

---

TyrProValGlySerGlnLeuProCysGluProGluProAspValAlaValLeuThrSer  
 301 ATACCCGGTAGGGTCGCAATTACCTTGCAGCCCCGAACCGGACGTGGCCGTGTGACGTC  
 TATGGGCCATCCAGCGTTAATGGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAG

---

MetLeuThrAspProSerHisIleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGly  
 361 CATGCTCACTGATCCCTCCCATATAACAGCAGAGGGCGGGCGAAGGTGGCGAGGGG  
 GTACGCTGACTAGGGAGGGTATATTGTCGTCTCGCCGGCCGCTTCCAACCGCTCCCC

---

SerProProSerValAlaSerSerSerAlaSerGlnLeuSerAlaProSerLeuLysAla  
 421 ATACCCCCCTCTGTGGCCAGCTCCCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGC  
 TAGTGGGGGAGACACCGGTGAGGAGCGGATCGGTGATAGGCGAGGTAGAGAGTTCCG

---

ThrCysThrAlaAsnHisAspSerProAsp  
 481 AACTTGCACCGCTAACCATGACTCCCCTGAT  
 TTGAACGTGGCGATTGGTACTGAGGGGACTA

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FIG. 19 Translation of DNA 8f

-----Overlap with 14c-----

1 SerSerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHis  
AGCTCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACTGCACCGCTAACCAT  
TCGAGGAGCCGATCGGTCGATAGGCGAGGTAGAGAGTTCCGTTGAACGTGGCGATTGGTA

61 AspSerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGly  
GACTCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGGCAGGAGATGGGCGGC  
CTGAGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCCGTCCTCTACCGCGC

121 AsnIleThrArgValGluSerGluAsnLysValValIleLeuAspSerPheAspProLeu  
AACATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGACTCCTTCGATCCGCTT  
TTGTAGTGGTCCCAACTCAGTCTTTTGTTCACCACTAGACCTGAGGAAGCTAGGCGAA

181 ValAlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArg  
GTGGCGGAGGAGGACGAGCGGAGATCTCCGTACCGCGAGAAATCCTGCGGAAGTCTCGG  
CACCGCCTCCTCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTAGGAAGCCTTCAGAGCC

241 ArgPheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGlu  
AGATTCCGCCAGGCCCTGCCGTTTGGGGCGGGCGGACTATAACCCCGCTAGTGGAG  
TCTAAGCGGGTCCGGGACGGGCAAACCCGCGCGGCTGATATTGGGGGGCGATCACCTC

301 ThrTrpLysLysProAspTyrGluProProValValHisGlyCysProLeuProProPro  
ACGTGGAAAAAGCCCGACTACGAACCACTGTGGTCCATGGCTGTCCGCTTCCACCTCCA  
TGCACCTTTTTCGGGCTGATGCTTGGTGGACACCAGGTACCGACAGGCGAAGGTGGAGGT

361 LysSerProProValPro  
AAGTCCCTCCTGTGCCG  
TTCAGGGGAGGACACGGC

FIG. 20 Translation of DNA 33f

1 ValTrpAlaArgProAspTyrAsnProProLeuValGluThrTrpLysLysProAspTyr  
CGTTTGGGGCGGGCCGACTATAACCCCGCTAGTGGAGACGTGGAAAAACCCGACTA  
GCAAACCCGCGCCGCTGATATTGGGGGGCGATCACCTCTGCACCTTTTTTGGGCTGAT

-----Overlap with 8f-----

61 GluProProValValHisGlyCysProLeuProProProLysSerProProValProPro  
CGAACCACTGTGGTCCATGGCTGCCGCTTCCACCTCCAAAGTCCCTCCTGTGCCTCC  
GCTTGGTGGACACCAGGTACCGACGGGCGAAGGTGGAGGTTTCAGGGGAGGACACGGAGG

121 ProArgLysLysArgThrValValLeuThrGluSerThrLeuSerThrAlaLeuAlaGlu  
GCCTCGGAAGAAGCGGACGGTGGTCTCACTGAATCAACCTATCTACTGCCTTGGCOGA  
CGGAGCCTTCTTCGCTGCCACAGGAGTGACTTAGTTGGGATAGATGAOOGAACCGGCT

181 LeuAlaThrArgSerPheGlySerSerSerThrSerGlyIleThrGlyAspAsnThrThr  
GCTCGCCACCAAGCTTTGGCAGCTCCTCAACTTCCGGCATTAAGGGCGACAATAAGAC  
CGAGCGGTGGTCTTCGAAACGTCGAGGAGTTGAAGGCCGTAATGCCCGCTGTTATGCTG

241 ThrSerSerGluProAlaProSerGlyCysProProAspSerAspAlaGluSerPhe  
AACATCCTCTGAGCCCGCCCTTCTGGCTGCCCGCCCGACTCCGACGCTGAGTCCCTTGC  
TTGTAGGAGACTCGGGCGGGGAAGACCGACGGGGGGGCTGAGGCTGCGACTCAGGAAACG

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FIG. 21 Translation of DNA 33g

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1  AlaSerArgSerPheGlySerSerSerThrSerGlyIleThrGlyAspAsnThrThrThr
   GCCTCCAGAAGCTTTGGCAGCTCCTCAACTCCGGCATACGGGOGACAATACGACAACA
   CGGAGGTCTTCGAAACCGTCGAGGAGTTGAAGGCGGTAATGCCCGCTGTATGCTGTGT
-----Overlap with 33f-----
61  SerSerGluProAlaProSerGlyCysProProAspSerAspAlaGluSerTyrSerSer
   TCCTCTGAGCCCGCCCTTCGGCTGCCCGCCGACTCCGACGCTGAGTCTATTCCTCC
   AGGAGACTCGGGCGGGGAAGACCGACGGGGGGCTGAGGCTGGACTCAGGATAAGGAGG
121  MetProProLeuGluGlyGluProGlyAspProAspLeuSerAspGlySerTrpSerThr
   ATGCCCCCCTGGAGGGGAGCCTGGGGATCCGGATCTTAGCGACGGGTCAATGGTCAACG
   TACGGGGGGACCTCCCTCGGACCCCTAGGCTAGAAATCGCTGCCAGTAACAGTTGG
181  ValSerSerGluAlaAsnAlaGluAspValValCysCysSerMetSerTyrSerTrpThr
   GTCAGTAGTGAGGCCAACCGCGGAGGATGTCGTGTGCTGCTCAATGCTTACTCTGGACA
   CAGTCATCACTCCGGTTGCGCCTCTACAGCACACGACGAGTTACAGAATGAGAACCTGT
241  GlyAlaLeuValThrProCysAlaAlaGluGluGlnLysLeuProIleAsnAlaLeuSer
   GCGCGCACTGTCACCCCGTGGCGCGGAAGAACAGAACTGCCCATCAATGCCTAAGC
   CCGCGTGAGCAGTGGGGCAGCGGGGCGCTTCTGTCTTTGACGGGTAGTTACGTGATTGC
301  AsnSerLeuLeuArgHisHisAsnLeuValTyrSerThrThrSerArgSer
   AACTCGTTGCTACGTCAACCAATTGGTGTATTCCACCACCTCAGCAGTG
   TTGAGCAACGATGCAGTGGTGTAAACCACATAAGGTGGTGGAGTGCGTCAC

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FIG. 22 Translation of DNA 7f

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1  GlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAlaHisAsnGlyLeuArg
   GGCACCTATGTTTATAACCATCTCACTCCTCTTCGGGACTGGGGCGACAAACGGCTTCGGA
   CCGTGATACAAATATTGGTAGAGTGAGGAGAAGCCCTGACCCGCGTGTTCGCGAACGCT
61  AspLeuAlaValAlaValGluProValValPheSerGlnMetGluThrLysLeuIleThr
   GATCTGGCCGTGGCTGTAGAGCCAGTCTCTTCTCCAAATGGAGACCAAGCTCATCAGC
   CTAGACCGGCACCGACATCTCGGTACAGAGAAGAGGGTTTACCTCTGGTTGAGTAGTGC
121  TrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeuProValSerAlaArg
   TGGGGGGCAGATACCGCGCGTGGCGGTGACATCATCAACGGCTTGCTGTTCGCGCCCGC
   ACCCCCCGTCTATGGCGGCGCACGCCACTGTAGTAGTTGCGGAACGGACAAAGCGGGCG
-----
181  ArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSerLysGlyTrpArgLeu
   AGGGGCGGGAGATACTGCTCGGGCCAGCCGATGGAATGGTCTCCAAGGGTTGGAGGTTG
   TCCCCGGCCCTCTATGACGAGCCCGGTGCGCTACCTTACCAGAGGTTCCTAACCTCCAAC
-----
241  LeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeuGlyCysIleIleThr
   CTGGCGCCCATCACGGCGTACGCCAGCAGACAAGGGGCTCCTAGGGTGATAATCACC
   GACCGCGGTAGTGCCGATGCGGGTCGTCGTTCCCGGAGGATCCCACGTATTAGTGG
-----Overlap with 7e-----
301  SerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGlnIleValSerThrAla
   AGCCTAATGGCCGGACAAAACCAAGTGGAGGGTGAGGTCCAGATTGTGTCAACTGCT
   TCGGATTGACCGGCCTGTTTTTGGTTACCTCCCACTCCAGGTCTAACACAGTTGACGA
-----
361  AlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrp
   GCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGG
   CGGGTTTGAAGGACCGTTGCACGTAGTTACCCACACGACC

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**FIG. 23** Translation of DNA 11b

GlyGlyValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyr  
 1 GGCGGTGTTGTTCTCGTCGGGTGATGGCGCTGACTCTGTCACCATATTACAAGCGCTAT  
 CCGCCACAACAAGAGCAGCCCACTACCGCGACTGAGACAGTGGTATAATGTTCCGCATA

IleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHis  
 61 ATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTCTGACCAGAGTGAAGCGCAACTGCAC  
 TAGTCGACCACGAACACCACCGAAGTCATAAAAGACTGGTCTCACCTTCGCGTTGACGTG

ValTrpIleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCys  
 121 GTGTGGATTCCCCCCTCAACGTCCGAGGGGGGCGCAGCCGTCATCTTACTCATGTGT  
 CACACCTAAGGGGGGAGTTGCAGGCTCCCCCGCGCTGCGGCAGTAGAATGAGTACACA

AlaValHisProThrLeuValPheAspIleThrLysLeuLeuLeuAlaValPheGlyPro  
 181 GCTGTACACCCGACTCTGGTATTGACATCACCAAATTGCTGCTGGCCGCTCTCGGACCC  
 CGACATGTGGGCTGAGACCATAAACTGTAGTGGTTTAACGACGACCGGCAGAAGCCTGGG

LeuTrpIleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeu  
 241 CTTTGGATTCTTCAAGCCAGTTTGCTTAAAGTACCCTACTTGTGCGCGTCCAAGGCCTT  
 GAAACCTAAGAAGTTCGGTCAAACGAATTTTCATGGGATGAAACACGCGCAGGTTCCGGAA

LeuArgPheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIle  
 301 CTCCGGTTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTGCAAATGGTCATC  
 GAGGCCAAGACGCGCAATCGGCCTTCTACTAGCCTCCGGTAATGCACGTTTACCAGTAG

-----  
 361 IleLysLeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeuArgAsp  
 ATTAAGTTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCACTCCTCTTCGGGAC  
 TAATTCAATCCCCGGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAGAAGCCCTG

-----Overlap with 7f -----  
 421 TrpAlaHisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPheSerGln  
 TGGGCGCACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTCGTCTTCTCCCAA  
 ACCCGCGTGTGCCGAACGCTCTAGACCGGCACCGACATCTCGGTCAGCAGAAGAGGGTT

-----  
 481 MetGluThrLysLeuIleThrTrpGly  
 ATGGAGACCAAGCTCATCACGTGGGGGGC  
 TACCTCTGGTTCGAGTAGTGACCCCCCG

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# FIG. 24 Translation of DNA 141

1 GluTyrValValLeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCysLeuTrp  
GGGAGTACGTCGTTCTCCTGTTCTGCTTGCGAGACGCGCGCTCTGCTCTGCTGTTG  
CCTCATGCAGCAAGAGGACAAGGAAGACGACGCTCTGCGCGCGAGACGAGGACGAACA

61 MetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAla  
GGATGATGCTACTCATATCCCAAGCGGAGCGGCTTGAGAGAACCTCGTAATACTTAATG  
CCTACTACGATGAGTATAGGGTTCGCTCCGCGCAACCTCTTGAGCATTATGAATTAC

121 AlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrp  
CAGCATCCCTGGCCGGGACGACGGTCTGTATCTCTCTGCTGTTCTTCTGCTTTGCAT  
GTCGTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGCACAAGAAGACGAAACGTA

181 TyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeu  
GGTATTGAAGGTAAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTGGCCTC  
CCATAAACATCCCATTCACCCACGGGCTCGCCAGATGTGGAAGATGCCCTACACCGGAG

241 LeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAla  
TCCTCTGCTCTGTTGGCGTTGCCCGAGCGGCGTACGCGCTGGACACGGAGGTGGCCG  
AGGAGGACGAGGACAACGCAACGGGTGCGCCGATGCGCGACCTGTGCTCCACCGGC

-----Overlap with 11b-----

301 SerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLys  
CGTCGTGTGGCGGTGTTGTTCTGCTCGGGTTGATGGCGCTGACTCTGTCAACATATTACA  
GCAGCACACCGCCACAACAAGAGCAGCCCACTACCGGACTGAGACAGTGGTATAATGT

361 ArgTyrIleSerTrpCysLeuTrpTrpLeuGln  
AGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGAA  
TCGCGATATAGTCGACCAACGACACCCACCGAAGTCTT

# FIG. 25 Translation of DNA 39c

1 ProAlaProSerGlyCysProProAspSerAspAlaGluSerTyrSerSerMetProPro  
CCAGCCCTTCTGCTGCCCCCGACTCCGACGCTGAGTCTTATTCCTCCATGCCCGCC  
GGTCGGGGAGACCGACGGGGGGCTGAGGCTGCGACTCAGGATAAGGAGGTACGGGGG

61 LeuGluGlyGluProGlyAspProAspLeuSerAspGlySerTrpSerThrValSerSer  
CTGGAGGGGGAGCCTGGGGATCCGGATCTTAGCGAGGGTCATGGTCAACAGTCAGTAGT  
GACCTCCCCCTCGACCCCTAGGCCTAGAAATCGCTGCCAGTACCAGTTGTCAGTCATCA

-----Overlap with 33g-----

121 GluAlaAsnAlaGluAspValValCysCysSerMetSerTyrSerTrpThrGlyAlaLeu  
GAGGCCAACGCGGAGGATGCTGTGCTGCTCAATGTCTTCTTGGACAGGCGCACTC  
CTCCGGTTGCGCCTCCTACAGCACACGAGGTACAGGATGAGAACCTGTCCGGTGAG

181 ValThrProCysAlaAlaGluGluGlnLysLeuProIleAsnAlaLeuSerAsnSerLeu  
GTCACCCCGTGGCGCGCGGAAGAACGAACTGCCCATCAATGCACTGAGCAACTCGTTG  
CAGTGGGGCAAGCGCGGCTTCTTGTCTTTGACGGGTAGTTACGTGACTCGTTGAGCAAC

241 LeuArgHisHisAsnLeuValTyrSerThrThrSerArgSerAlaCysGlnArgGlnLys  
CTACGTCACCACAATTTGGTGTATTCCACCACCTCAGCAGTGTCTGCCAAAGGCAGAAG  
GATGCAGTGGTGTAAACACATAAGGTGGTGGAGTGCGTACGAAACGGTTTCCGTCTTC

301 LysValThrPheAspArgLeuGlnValLeuAspSerHisTyrGlnAspValLeuLysGlu  
AAAGTCACATTTGACAGACTGCAAGTTCTGGACAGCCATTACCAGGACGTACTCAAGGAG  
TTTCAGTGTAAGTGTCTGACGTTCAAGACCTGTGGTAATGGTCTGCATGAGTTCTCTC

361 ValLysAlaAlaAlaSerLysValLysAlaAsnPhe  
GTTAAAGCAGCGGCTCAAAGTGAAGCGTAACCTC  
CAATTCGTGCGCGAGTTTCACTTCCGATTGAAG

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**FIG. 26-1** COMBINED ORF OF DNAs

141/11b/7f/7e/8h/33c/40b/37b/35/36/81/32/33b/25c/14c/8f/33f/33g/39c

GluTyrValValLeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCysLeuTrp  
 1 GGGAGTACGTCTCTCTCTGTTCTCTGCTTGACAGACGCGCGCTCTGCTCCTGCTTGT  
 CCTCATGTCAGCAAGAGGACAAGGAAGACGAACGTCTGCGCGCAGACGAGGACGAACA  
  
 MetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAla  
 61 GGATGATGCTACTCATATCCCAAGCGGAGCGGCTTTGGAGAACCTCGTAATACTTAATG  
 CCTACTACGATGAGTATAGGGTTGCTCCGCGGAAACCTCTTGGAGCATTATGAATTAC  
  
 AlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrp  
 121 CAGCATCCCTGGCGGGACGCACGGCTCTGTATCCTTCTCTGCTTCTCTGCTTGGCAT  
 GTGCTAGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGCACAAGAAGACGAAACGTA  
  
 TyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeu  
 181 GGTATTGTAAGGTAAGTGGGTGCCCGAGCGGTCTACACCTTCTACCGGATGTGGCTC  
 CCATAAATTCCCATTCACCCACGGGCTCGCCAGATGTGGAAGATGCCCTACACGGAG  
  
 LeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAla  
 241 TCCTCCTGCTCCTGTTGGCGTTGCCCGAGCGGCGTACGCGCTGGACACGGAGGTGGCCG  
 AGGAGGACGAGGACAACCGCAACGGGGTCCCGCATGCGCGACCTGTGCCTCCACCGGC  
  
 SerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLys  
 301 CGTCGTGTGGCGGTGTGTTCTCGTCCGGTTGATGGCGCTGACTCTGTCAACCATATTACA  
 GCAGCACACGCCACAACAAGAGCAGCCCACTACCGGACTGAGACAGTGGTATAATGT  
  
 ArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGln  
 361 AGCGCTATATCAGCTGGTGTCTTGGTGGCTTCACTATTCTCTGACAGAGTGAAGCGC  
 TCGCGATATAGTCGACCAGAACACCACCGAAGTCATAAAGACTGGTCTCACCTTCGCG  
  
 LeuHisValTrpIleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeu  
 421 AACTGCACGTGTGGATTCCCCCTCAACGTCCGAGGGGGCGCGACGCGTCACTCTTAC  
 TTGACGTGCACACCTAAGGGGGGAGTTGCAGGCTCCCCCGCGCTGCGGCAGTAGAATG  
  
 MetCysAlaValHisProThrLeuValPheAspIleThrLysLeuLeuLeuAlaValPhe  
 481 TCATGTGTGCTGTACACCGACTCTGTTATTTGACATCACCAAATTGCTGTGCGCGTCT  
 AGTACACAGCATGTGGCTGAGACCATAACTGTAGTGGTTAAAGACGACCGGCAGA  
  
 GlyProLeuTrpIleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGln  
 541 TCGGACCCCTTTGGATTCTCAAGCCAGTTTGCTTAAAGTACCTACTTTGTGCGCGTCC  
 AGCCTGGGGAACCTAAGAAGTTCGGTCAAACGAATTTATGGGATGAACACGCGCAGG  
  
 GlyLeuLeuArgPheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMet  
 601 AAGGCCCTTCTCCGTTCTGCGGTTAGCGCGGAAGATGATCGGAGGCCATTACGTGCAAA  
 TTCCGGAAGAGGCCAAGACGCGCAATGCGCCTCTACTAGCCTCCGGTAATGCAGCTTT  
  
 ValIleIleLysLeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeu  
 661 TGGTCATCATTAAAGTTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCACTCCTC  
 ACCAGTAGTAATTCAATCCCGCGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAG  
  
 ArgAspTrpAlaHisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPhe  
 721 TTCGGGACTGGGCGCACAAAGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTGTGTCT  
 AAGCCCTGACCCGCGTGTGCGGAACGCTTAGACCGGCACCGACATCTGGTTCAGCAGA  
  
 SerGlnMetGluThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIle  
 781 TCCTCCAAATGGAGACCAAGCTCATCAGTGGGGGGCAGATACCGCCGCTGGGGTACCA  
 AGAGGGTTTACCTCTGGTTCGAGTAGTGACCCCCCGTCTATGGCGGCGCACGCCACTGT  
  
 IleAsnGlyLeuProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAsp  
 841 TCATCAACGGCTTGCCGTCTTCGCGCGAGGGGCGGGGAGATACTGCTCGGGCCAGCCG  
 AGTAGTTGCCGAACGGACAAAGGCGGGCGTCCCGGCCCTCTATGACGAGCCCGTCCGC  
  
 GlyMetValSerLysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThr

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901 ATGGAATGGTCTCCAAGGGGTGGAGGTTGCTGGCGCC@ATCACGGCGTACGCCAGCAGA  
 TACCTTACCAGAGGTTCCTCCACCTCCAACGACCGCGG@TAGTGCGCATGCGGGTGGTCT  
  
 ArgGlyLeuLeuGlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGlu  
 961 CAAGGGGCTCTAGGGTGCTAATCACCAGCCTAAGTGGCGGGACAAAACCAAGTGG  
 GTTCCCGGAGGATCCACGTATTAGTGGTGGATTGACCGGCCCTGTTTGGTTCCACC  
  
 GlyGluValGlnIleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGly  
 1021 AGGGTGAGGTCCAGATTGTCTCAACTGCTGCCAAG@TTCCTGGCAACGTGCATCAATG  
 TCCACTCCAGGTCTAACACAGTTGACGACGGGTTTGG@AGGACCGTTGCACTAGTTAC  
  
 ValCysTrpThrValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyPro  
 1081 GGGTGTGCTGGACTGTCTACCAAGGGGCGGAACGAG@ACCATCGCTACCCAAGGGTC  
 CCCACACGACCTGACAGATGGTCCCCCGGCTTCTCTG@TGGTAGCGCAGTGGGTTCCACG  
  
 ValIleGlnMetTyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGly  
 1141 CTGTCTATCCAGATGTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTCCGCAAG  
 GACAGTAGGTCTACATATGGTTACATCTGGTTCTGGARCA@CCGACCGGGGAGGGGCTTC  
  
 SerArgSerLeuThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHis  
 1201 GTAGCGCTCATTGACACCTGCACTTGGCGCTCTCTG@GACCTTTACCTGGTCAAGAGGC  
 CATCGGCGAGTAACTTGGGACGTGAACGCGAGGAG@CTGGAAATGGAACGAGTGGTCTCG  
  
 AlaAspValIleProValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArg  
 1261 ACGCGATGTCTATCCCGTGGCGCGGGGTGATAG@AGGGGCGAGCTGCTGTGCGCCC  
 TGCGGCTACAGTAAAGGGCAGCGCGCGCGCCCACTATG@TCCCGTCCGACGACAGCGGGG  
  
 ProIleSerTyrLeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAla  
 1321 GGCCCATTTCTACTTGAAGGCTCTCTCGGGGGTCCGCTGTGTGTC@CGCGGGGCGAGC  
 CCGGTAAGGATGAACTTTCCGAGGAGCCCC@AGGGGACACACGGGGGCGCCCGTGC  
  
 ValGlyIlePheArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIle  
 1381 CCGTGGGCATATTTAGGGCGCGGTGTGCACCGTGGAGTGGCTAAGGCGGTGGACTTGA  
 GGCACCGTATAAATCCCGGCGCCACAGTGGGCACTTCA@CGGATTCCGCCACTGAAT  
  
 ProValGluAsnLeuGluThrThrMetArgSerProValPheThrAspAsnSerSerPro  
 1441 TCCCTGTGGAGAACCTAGAGACAACCATGAGGT@CCCGGTTTCACGGATAACTCTCTC  
 AGGGACACCTCTTGGATCTCTGTGCTACTCCAGGG@CCACAAGTGCTATTGAGGAGAG  
  
 ProValValProGlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLys  
 1501 CACCACTAGTGCCTCCAGAGCTTCCAGGTGGCTCA@CTCATGCTCCACAGGCGAGGGCA  
 GTGGTCATCACGGGTCTCGAAGGTCCACGAGTGGAGGTACGAGGGTGTCCGTCCCGT  
  
 SerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnPro  
 1561 AAAGCACCAGGTCCCGCTGCATATGCAGCTCAGGG@CTATAAGGTGCTAGTACTCAACC  
 TTTCTGGTTCCAGGGCGAAGTATACGTGAGTCCG@ATATTCCAGATCATGATTGG  
  
 SerValAlaAlaThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspPro  
 1621 CCTCTGTGTGCAACACTGGGCTTTGGTGTCTACATGTC@AAGGCTCATGGGATCGATC  
 GGAGACAACGACGTTGTGACCCGAAACACGAATGTACAGGTCCGAGTACCTAGCTAG  
  
 AsnIleArgThrGlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyr  
 1681 CTAACATCAGGACCGGGTGAGAACAAATTACCACTGGCAGCCCCATCAGTACTCCACCT  
 GATTGTAGTCTGGCCCCACTCTGTGTTAATGGTGA@CGTCCGGGTAGTGATGAGGTGGA  
  
 GlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleCysAsp  
 1741 ACGGCAAGTTCTTGGCGAGCGGGTGTCTCGGGGGG@CGCTTATGACATAATAATTGTG  
 TGCCGTTCAAGGAACGGCTGCCGCCACGAGCC@CCCGGAATACTGTATTATTAACAC  
  
 GluCysHisSerThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAla  
 1801 ACGAGTGCCACTCCACGATGCCACATCCATCTTGGG@CATCGGCAGTGTCTTGAACCAAG  
 TGCTCACGGTGAGGTGCCTACGGTGTAGGTAGAAC@CGTAGCCGTGACAGGAAGTGGTTC  
  
 GluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThr  
 1861 CAGAGACTGCCGGGGCGAGACTGTTGTGCTCGCC@ACCGCCACCCCTCCGGGCTCCGTCA  
 GTCTCTGACGCCCCGCTCTGACCAACACGAGCGGTGGCGGTGGGAGGGCCGAGGCAGT

FIG. 26-2.

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ValProHisProAsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyr  
 1921 CTGTGCCCCATCCCAACATGAGGAGGTGCTCTGTCCACCACGGAGAGATCCCTTTT  
 GACACGGGGTAGGGTGTAGCTCCTCCAACGAGACAGGTGGTGGCTCTCTAGGGA AAAA

GlyLysAlaIleProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSer  
 1981 ACGGCAAGGCTATCCCOCTGGAAGTAATCAAGGGGGGAGACATCTCATCTTCTGTCTAT  
 TGGCGTTCCGATAGGGGAGCTTCATTAGTTCCOCCCCCTCTGTAGAGTAGAAGACAGTAA

LysLysLysCysAspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAla  
 2041 CAAAGAAGAAGTGGCAAGAACTGCGCGCAAAGCTGGTGCATTGGGCATCAATGCCGTGG  
 GTTCTCTCTTCAAGCTGCTTGAGCGGGGTTTCGACCAGCGTAACCCGTAGTTACGGCACC

TyrTyrArgGlyLeuAspValSerValIleProThrSerGlyAspValValValValAla  
 2101 CCTACTACGCGGCTCTTGACGTGTCCGTATCCCGACCAGCGCGATGTTGTGTGTGTGG  
 GGATGATGGCGCCAGAACTGCACAGGCAGTAGGGCTGGTGGCGCTACAACAGCAGCACC

ThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThr  
 2161 CAACCGATGCCCTCATGACCGGCTATACCGCGAGCTTCGACTCGGTGATAGACTGCAATA  
 GTTGGCTACGGGAGTACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTAT

CysValThrGlnThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThr  
 2221 CGTGTGTCAACGACAGTCGATTTCAGCCTTGACCTACCTTCACCATGAGACAATCA  
 GCACACAGTGGGTCTGTCTAGCTAAAGTCGGAACCTGGGATGGAAGTGGTAACTCTGTTAGT

LeuProGlnAspAlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysPro  
 2281 CGCTCCCCAGGATGCTGTCTCCGCACTCAACCTCGGGGAGGAGCTGGCAGGGGGAAGC  
 GCGAGGGGGTCTACGACAGAGGGCGTGAGTTGCAGCCCGTCTGACCGTCCCCCTCG

GlyIleTyrArgPheValAlaProGlyGluArgProSerGlyMetPheAspSerSerVal  
 2341 CAGGCATCTACAGATTGTGGCAACGGGGGAGCGCCCTCCGCGATGTTGACTCGTCCG  
 GTCCGTAGATGTCTAAACACCGTGGCCCTCGCGGGAGGCGGTACAAGCTGAGCAGGC

LeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThr  
 2401 TCCTCTGTGAGTGTATGAAGCAGGCTGTGCTTGGTATGAGCTCACGCCCGCGAGACTA  
 AGGAGACACTCACGATACTGCGTCCGACACGAACCATCTCGAGTGGGGCGGCTCTGAT

ValArgLeuArgAlaTyrMetAsnThrProGlyLeuProValCysGlnAspHisLeuGlu  
 2461 CAGTTAGGCTACGAGCGTACATGAACACCCCGGGCTTCCCGTGTGCCAGGACCATCTTG  
 GTCAATCCGATGCTCGCATGTACTTGTGGGGCCCGAAGGGCACACGTCCTGGTAGAAC

PheTrpGluGlyValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThr  
 2521 AATTTTGGAGGGCGTCTTTACAGGCTCACTCATATAGATGCCACTTTCTATCCAGA  
 TTA AAACCTCCCGCAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAGATAGGGTCT

LysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCysAlaArg  
 2581 CAAAGCAGAGTGGGAGAACCTTCTTACCTGGTAGCGTACCAAGCCACCGTGTCCGCTA  
 GTTTCGTCTCACCCCTCTTGAAGGAATGAACATCGCATGGTTCGGTGGCACACCGCAT

AlaGlnAlaProProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysPro  
 2641 GGGCTCAAGCCCTCCCCCATCGTGGGACAGATGTGGAAGTCTTTGATTCCGCTCAAGC  
 CCCGAGTTCGGGAGGGGGTAGCACCTGGTCTACACCTTCACAACTAAGCGGAGTTCG

ThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGluIleThr  
 2701 CCACCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCTGTTCAGAAATGAATCA  
 GGTGGGAGGTACCCGTTGTGGGACGATATGCTGTACCCGCGACAAGTCTTACTTTAGT

LeuThrHisProValThrLysTyrIleMetThrCysMetSerAlaAspLeuGluValVal  
 2761 CCCTGACGCAACCCAGTCACCAATACATCATGACATGTCGGCCGACCTGGAGGTCCG  
 GGGACTGCGTGGGTCACTGGTTTATGTAGTACTGTACGTACAGCCGCTGGACCTCAGC

ThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCysLeuSer  
 2821 TCACGAGCACCTGGGTGCTCGTTGGCGGCTCTGGCTGCTTTGGCCGCTATTGCCCTGT  
 AGTGCTCGTGACCCACGAGCAACCGCCGAGGACCGAAGAACCGGCGCATACCGGACA

ThrGlyCysValValIleValGlyArgValValLeuSerGlyLysProAlaIleIlePro

FIG. 26-3

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2881 CAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTGTGCCGGAAGCCGGCAATCATAC  
GTTGTCCGACGCCAGTATCACCGTCCAGCAGAACAGGCCCTTGGCCGTTAGTATG

2941 AspArgGluValLeuTyrArgGluPheAspGluMetGluGluCysSerGlnHisLeuPro  
CTGACAGGGAGTCTCTACCGAGAGTTCGATGAGATGGAAGAGTGTCTCAGCACTTAC  
GACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTCTCAGGAGTGTGTAATG

3001 TyrIleGluGlnGlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeu  
CGTACATCGAGCAAGGGATGATGCTCGCGAGGAGTTCAGCAGAACGGCCCTCGGCCCTCC  
GCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCGTCTCCGGGAGCCGGAGG

3061 GlnThrAlaSerArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLys  
TGCAGACCGGCTCCCGTCAGGCAGAGGTTATCGCCCTGCTGTCCAGACCAACTGGCAAA  
ACGCTCGGCGCAGGCAGTCCGTCTCCAATAGCGGGACGACAGGTCTGGTTGACGGTTT

3121 LeuGluThrPheTrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAla  
AACTCGAGACCTTCTGGGCGAAGCATATGTGGAACCTTCATCAGTGGGATACAATACTTGG  
TTGAGCTCTGGAAGACCGCTTGTATACACCTTGAAGTAGTCACCTATGTTATGAACC

3181 GlyLeuSerThrLeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAla  
CGGGCTGTCAACGCTGCCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACAGCTG  
GCCCGAACAGTTGCGAAGGACCATGGGGCGGTAAAGTAAGTAACTACGAAAATGTGAC

3241 ValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGlyTrpVal  
CTGTCAACAGCCCACTAACCACTAGCCAAACCCCTCTCTTCAACATATGGGGGGGTGGG  
GACAGTGGTGGGTGATTGGTGTATCGGTTTGGGAGGAGAAGTTGTATAACCCCCCCACCC

3301 AlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeuAlaGly  
TGGCTGCCAGCTCGCCGCCCGCGTCCGCTACTGCCCTTGTGGGCGCTGGCTTAGCTG  
ACCGACGGGTGAGCGGGGGGGCCACGCGATGAAGAAACACCCGCGACCGAATCGAC

3361 AlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGly  
GGCCGCCCATCGGCAGTGTGGACTGGGGAAGGTCCTCATAGACATCTTGCAGGGTATG  
CGCGCGGTAGCCGTCACAACTGACCCCTTCAGGAGTATCTGTAGGAACGTCCCATAC

3421 AlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThr  
GGCGGGCGTGGCGGAGCTCTTGTGGCATTCAAGATCATGACGGGTGAGGTCCCTCCCA  
CGCGCCCGCACCGCCTCGAGAACACGTAAGTTCTACTACTGCCACTCCAGGGGAGGT

3481 GluAspLeuValAsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyVal  
CGGAGGACCTGGTCAATCTACTGCCCGCATCTCTCGCCCGAGCCCTCGTAGTCGGCG  
GCCTCTGGACCACTTAGATGACGGGCGGTAGGAGAGCGGGCTCGGGAGCATCAGCGCG

3541 ValCysAlaAlaIleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnTrpMet  
TGGTCTGTGCAGCAATACTGCGCGGCAAGTTGGCCCGGGCGAGGGGCGAGTGCAGTGA  
ACCAGACAGTCTGTATGACCGCGCGTGCACCGGGCCGCTCCCGCTCAGTCACTACCT

3601 AsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValPro  
TGAACCGGCTGATAGCCTTCGCTCCCGGGGAACCATGTTTCCCCACGCACTACGTGC  
ACTTGGCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGTGGTGATGCACG

3661 GluSerAspAlaAlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeu  
CGGAGAGCGATGCAGCTGCCCGGCTACTGCCATACTCAGCAGCCTCACTGTAAACCCAGC  
GCCTCTCGCTACGTGACGGGCGCAGTGACGGTATGAGTGTGCGAGTGACATTGGGTGG

3721 LeuArgArgLeuHisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrp  
TCTGAGGCGACTGCACCACTGGATAAGCTCGGAGTGTACCACTCCATGCTCGGGTTCT  
AGGACTCCGCTGACGTGGTCACTATTGAGCCTCACATGGTGGGTACGAGGCCAAGGA

3781 LeuArgAspIleTrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLys  
GGCTAAGGGACATCTGGGACTGGATATCGAGGTGTTGAGCGACTTTAAGACTGGCTAA  
CCGATTCCCTGTAGACCTGACCTATAGCTCCACAACCTGGCTGAATTTCTGACCGATT

3841 AlaLysLeuMetProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLys  
AAGCTAAGCTCATGCCACAGCTGCCTGGATCCCTTTGTGTCTGCCAGCGGGGTATA  
TTCGATTGAGTACGGTGTGACGGACCTAGGGGAACACAGGACGGTGGCGCCATAT

FIG. 26-4

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GlyValTrpArgValAspGlyIleMetHisThrArgCysHisCysGlyAlaGluIleThr  
 3901 AGGGGGTCTGGCGAGTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAGATCA  
 TCCCCAGACCGCTCAGCTGCCGTAGTACGTGTGAGGACGGTGACACCTGACACTCTAGT

GlyHisValLysAsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrp  
 3961 CTGGACATGTCAAAAACGGGACGATGAGGATCGTCGGTCTAGGACCTGCAGGAACATGT  
 GACCTGTACAGTTTTTGCCCTGCTACTCTAGCAGCCAGGATCTGGAGCTCCTGTGACA

SerGlyThrPheProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaPro  
 4021 GGAGTGGGACCTTCCCCATTAAAGCCTACACACGGGCCCCCTGTACCCCCCTTCTGCGC  
 CCTCACCTGGAGGGGTAAATTACGGATGTGGTGCCCGGGGACATGGGGGAAGGACGGG

AsnTyrThrPheAlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnVal  
 4081 CGAATACACCTTCGGCTATGAGGGGTGTCTCGAGAGGAATATGTGGAGATAAGGCAGG  
 GCTTGATGTGAAGCGGATACCTCCACAGAGCTCTCTTATACACCTCTATTCCGTCC

GlyAspPheHisTyrValThrGlyMetThrThrAspAsnLeuLysCysProCysGlnVal  
 4141 TGGGGGACTTCCACTAGCTGACGGGTATGACTACTGACAATCTCAAATGCCCGTGCCAGG  
 ACCCCCTGAAGGTGATGCACTGCCATCTGATGACTGTAGAGTTTACGGGCACGGTCC

ProSerProGluPhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProPro  
 4201 TCCCATCGCCCGAATTTTTCACAGAAATGGACGGGGTGGCGCTACATAGGTTTGGCGCCC  
 AGGGTAGCGGGCTTAAAAGTGTCTTAACCTGCCCCACGGGATGTATCAAACGGGGG

CysLysProLeuLeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProVal  
 4261 CCTGCAAGCCCTTGTCTCGGGAGGAGGTATCATTCAGAGTAGGACTCCAGGAATACCCGG  
 GGACGTTCGGGAACGACGCCCTCTCCATAGTAAGTCTCATCTGAGGTGCTTATGGGCC

GlySerGlnLeuProCysGluProGluProAspValAlaValLeuThrSerMetLeuThr  
 4321 TAGGGTCGCAATTACCTTCGAGCGCCGAGACGAGCTGGCCGTGTGACGTGCATGCTCA  
 ATCCAGCGTTAATGGAACGCTCGGGCTTGGCTGCACCGGCACAACTGCAGGTACGAGT

AspProSerHisIleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProPro  
 4381 CTGATCCCTCCATATAACAGCAGAGCGCGCGCGAAGGTTGGCGAGGGATCACCCC  
 GACTAGGGAGGGTATATGTCTCTCGCGCGCCCGCTTCCAAACGCTCCCTAGTGGGG

SerValAlaSerSerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThr  
 4441 CCTCTGTGGCAGCTCTCTGGCTAGCCAGCTATCGCTCCATCTCTCAAGGCACCTTGCA  
 GGAGACACCGTTCGAGGAGCGATCGGTTCGATAGGCGAGGTAGAGAGTTCCGTTGAACGT

AlaAsnHisAspSerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGlu  
 4501 CCGCTAACCATGACTCCCTGATGCTGAGCTCATAGAGGCCAACCTCTATGGAGGCAGG  
 GCGGATTGGTACTGAGGGGACTAAGCTCGAGTATCTCCGTTGGAGGATACTCCGTCC

MetGlyGlyAsnIleThrArgValGluSerGluAsnLysValValIleLeuAspSerPhe  
 4561 AGATGGGCGGCAACATCACCAGGGTTGAGTCAAGAAACAAAGTGGTGATTCTGGACTCCT  
 TCTACCGCGCTTGTAGTGGTCCCACTCAGTCTTTTGTTCACCACTAAGACCTGAGGA

AspProLeuValAlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArg  
 4621 TCGATCCGCTTGTGGCGGAGGAGGACGAGCGGAGATCTCGTACCCGAGAAATCCTGC  
 AGCTAGGCGAACACCGCTCTCTCTCGCTCTAGAGGCATGGGGTCTTTAGGACG

LysSerArgArgPheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProPro  
 4681 GGAAGTCTCGGAGATCGCCCGAGCCCTGCCGTTTGGGCGCGCCGAGCTATAACCCCC  
 CCTTCAGAGCTCTAAGCGGGTCCGGACGGGCAACCCCGCGCGGCTGATATTGGGGG

LeuValGluThrTrpLysLysProAspTyrGluProProValValHisGlyCysProLeu  
 4741 CGCTAGTGGAGACGTGGAAGAGCCCGACTAAGAACCACTGTGGTGCATGGCTGTCCGC  
 GCGATCACCTCTGCACCTTTTGGGGCTGATGCTTGGTGGACACCAGGTACGACAGGCG

ProProProLysSerProProValProProProArgLysLysArgThrValValLeuThr  
 4801 TTCCACCTCCAAAGTCCCTCTGTGCTCGCCCTCGGAGAGAGCGGACGGTGGTCTCTCA  
 AAGGTGGAGGTTTCAGGGGAGGACACGAGGGGAGCCCTTCTGCGCTGCCACAGGAGT

GluSerThrLeuSerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSer

FIG. 26-5

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4861 CTGAATCAACCCCTATCTACTGCCTTGGCGAGCTCGCCACCAGAAGCTTTGGCAGCTCCT  
 GACTTAGTTGGGATAGATGACGGAACCGGCTCGAGCGGTGGTCTTCGAAACCGTCGAGGA  
  
 ThrSerGlyIleThrGlyAspAsnThrThrThrSerSerGluProAlaProSerGlyCys  
 4921 CAACTTCGGCATTACGGGCGACAATACGACAACATCCTCTGAGCCGCCCCCTCTGGCT  
 GTTGAAGGCCGTAAATGCCCGCTGTTATGCTGTTGTAGGAGACTCGGGCGGGGAAGACCGA  
  
 ProProAspSerAspAlaGluSerTyrSerSerMetProProLeuGluGlyGluProGly  
 4981 GCGCCCCGACTCCGACGCTGAGTCCTATTCTCCATGCCCCCCTGGAGGGGGAGCCTG  
 CGGGGGGGCTGAGGCTGCGACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCTGGAC  
  
 AspProAspLeuSerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAsp  
 5041 GGGATCCGGATCTTAGCGAAGGGTCATGTCACCGGTCAGTAGTGAGGCCAACGCGGAGG  
 CCTTAGGCCTAGAATCGCTGCCAGTACCAGTTGCCAGTCATCACTCCGGTTGCGCTCC  
  
 ValValCysCysSerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAla  
 5101 ATGTCGTGTGCTGCTCAATGCTTACTCTTGGACAGGCGCACTCGTCACCCGTTGCGCG  
 TACAGCACACGACGATTACAGAATGAGAACCTGTCCGCGTGAGCAGTGGGGCACGCGG  
  
 GluGluGlnLysLeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHisAsnLeu  
 5161 CGGAAGAACAGAACTGCCATCAATGCACTAAGCAACTCGTTGCTACGTCAACCAATT  
 GCCTTCTTGCTCTTTGACGGGTAGTTACGTGATTGTTGAGCAACGATGCAGTGGTGTAA  
  
 ValTyrSerThrThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArg  
 5221 TGGTGATTCACCACTCAACGAGTGTGCTTGCCAAAGGCAGAAGAAAGTCACATTTGACA  
 ACCACATAAGGTGGTGGAGTGGTCAAGAACGGTTTCGCTCTTCTTCAGTGTAACCTGT  
  
 LeuGlnValLeuAspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaAlaSer  
 5281 GACTGCAAGTTCTGGACAGCCATTACAGGACGTACTCAAGGAGGTAAAGCAGCGGCGT  
 CTGACGTTCAAGACCTGTCGGTAATGGTCTGCATGAGTTCCTCCAATTTGTCGCGCGA  
  
 LysValLysAlaAsnLeu  
 5341 CAAAAGTGAAGGCTAACTTG  
 GTTTTCACTTCOGATTGAAC

FIG. 26-6

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**FIG. 27** Translation of DNA 12f

IlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsn  
 1 CCATATTTTAAATCAGGATGTACGTGGGAGGGGTGGAACACAGGCTGGAAGCTGCCTGCA  
 GGTATAAATTTTAGTCTACATGCACCTCCCCAGCTTGTGTCCGACCTTCGACGGACGT

TrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeu  
 61 ACTGGACCGGGGCGAACGTTGCGATCTGGAAGACAGGGACAGGTCGAGCTCAGCCCGT  
 TGACCTGCGCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGGCTCGAGTCGGGCA

LeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeu  
 121 TACTGCTGACCACTACACAGTGGCAGGTCTCCGTGTTCTTCAACCCCTACCAAGCT  
 ATGACGACTGGTGATGTGTACCGTCCAGGAGGGCACAAGGAAGTGTGGGATGGTCGGA

SerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyVal  
 181 TGTCCACCGGCTCATCCACCTCCACCAGAACATTGTGGACGTGCAGTACTGTACGGGG  
 ACAGGTGGCCCGAGTAGGTGGAGGTGGTCTTGTAAACCTGCAGTCATGAACATGCCCC

-----  
 GlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeu  
 241 TGGGGTCAAGCATCGCGTCTGGGCCATTAACTGGGAGTACGTGTTCTCCTGTTCTTC  
 ACCCCAGTTCGTAGCGCAGGACCCGGTAATTCACCCTCATGCAGCAAGAGGACAAGGAAG

-----  
 LeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGlu  
 301 TGCTTGACAGACGCGCGTCTGCTCCTGCTTGATGATGCTACTCATATCCAAGCGG  
 ACGAACGTCTGCGCGCGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTCGCC

-----Overlap with 141-----  
 AlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeu  
 361 AGGCGGCTTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGCGCGGGACGCACGGTC  
 TCCGCCGAACCTCTTGGAGCATTATGAATTACGTCTAGGGACCGGCCCTGCGTGCCAG

-----  
 Val  
 421 TTGTATC  
 AACATAG

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**FIG. 28** Translation of DNA 35f

-----Overlap with 39c-----

1 LeuLysGluValLysAlaAlaAlaSerLysValLysAlaAsnLeuSerValGluGlu  
 TGCTCAAGGAGGTAAAGCAGCGCGTCAAAGTGAAGGCTAACTTGCTATCCGTAGAGG  
 ACGAGTTCCCAATTTCTGCGCGCAGTTTTCACCTCCGATTGAACGATAGGCATCTCC

61 AlaCysSerLeuThrProProHisSerAlaLysSerLysPheGlyTyrGlyAlaLysAsp  
 AAGCTTGACGCTGACGCCCCACACTCAGCCAAATCCAAGTTTGGTTATGGGGCAAAG  
 TTCGAACGTCGGACTGCGGGGTGTGAGTCGGTTTAGGTTCAAACCAATACCCCGTTTC

121 ValArgCysHisAlaArgLysAlaValThrHisIleAsnSerValTrpLysAspLeuLeu  
 ACGTCCGTTGCCATGCCAGAAAGGCGTAACCCACATCAACTCCGTGTGGAAAGACCTTC  
 TGCAGGCAACGGTACGGTCTTCCGGCATTGGGTGTAGTTGAGGCACACCTTCTGGAAG

181 GluAspAsnValThrProIleAspThrThrIleMetAlaLysAsnGluValPheCysVal  
 TGGAAGACAATGTAACACCAATAGACACTACCATCATGGCTAAGAACGAGGTTTCTGCG  
 ACCTTCTGTTACATTGTGGTTATCTGTGATGGTAGTACCGATTCTTGCTCCAAAAGACGC

241 GlnProGluLysGlyGlyArgLysProAlaArgLeuIleValPheProAspLeuGlyVal  
 TTCAGCCTGAGAAGGGGGTTCGTAAGCCAGCTCGTCTCATCGTGTCCCCGATCTGGGCG  
 AAGTCGGACTCTTCCCCCAGCATTCGGTCGAGCAGAGTAGCACAAAGGGGCTAGACCCGC

301 ArgValCysGluLysMetAlaLeuTyrAspValValThrLysLeuProLeuAlaValMet  
 TCGCGGTGTGCGAAAAGATGGCTTTGTACGACGTGGTTACAAAGCTCCCTTGGCCGTGA  
 ACGCGCACACGCTTTCTACCGAAACATGCTGCACCAATGTTTCGAGGGGAACCGGCACT

361 GlySerSerTyrGlyPheGlnTyrSerProGlyGlnArgValGluPheLeuValGlnAla  
 TGGGAAGCTCCTACGGATTCCAATACTACCAGGACAGCGGGTTGAATTCCTCGTGCAAG  
 ACCCTTCGAGGATGCCTAAGGTTATGAGTGGTCTGTCGCCAACTTAAGGAGCACGTTT

421 TrpLysSerLysLysThrProMetGlyPheSerTyrAspThrArgCysPheAspSerThr  
 CGTGGAAGTCCAAGAAAACCCCAATGGGGTTCCTGATGATACCCGCTGCTTTGACTCCA  
 GCACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTATGGGCGACGAACTGAGGT

481 ValThrGluSerAspIleArgThrGluGluAla  
 CAGTCACTGAGAGCGACATCCGTACGGAGGAGGCA  
 GTCAGTGAATCTCGCTGTAGGCATGCTCCTCCGT

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**FIG. 29** Translation of DNA 19g

```

-----
1  GluPheLeuValGlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAspThr
   GAATTCCTCGTGCAAGCGTGAAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGATACC
   CTTAAGGAGCACGTTTCGACCTTCAGGTTCTTTGGGGTTACCCAAGAGCATACTATGG
-----Overlap with 35f-----
61  ArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyrGln
   CGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGGCAATCTACCAA
   GCGACGAAACTGAGGTGTCAGTGACTCTCGCTGTAGGCATGCCTCCTCGTTAGATGGTT
121  CysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeuTyr
   TGTGTGACCTCGACCCCCAAGCCCGGTGGCCATCAAGTCCCTCACCAGAGGGCTTTAT
   ACAACACTGGAGCTGGGGGTTCCGGCGCACCGGTAGTTCAGGGAGTGGCTCTCCGAAATA
181  ValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArgAla
   GTTGGGGGCCCTCTTACCAATTCAAGGGGGGAGAACTGCGGCTATCGCAGGTGCCGCGCG
   CAACCCCGGGGAGAATGGTTAAGTTCCCCCTCTTGACGCCGATAGCGTCCACGGCGCGC
241  SerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArgAla
   AGCGGCGTACTGACAACCTAGCTGTGGTAACACCCCTCACTTGCTACATCAAGGCCCGGGCA
   TCGCCGATGACTGTTGATCGACACCATTGTGGGAGTGAACGATGTAGTTCGGGGCCCGT
301  AlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeuVal
   GCCTGTCGAGCCGCAGGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTAGTC
   CGGACAGCTCGGCGTCCCGAGGTCTTGACGTGGTACGAGCACACCGCTGCTGAATCAG
361  ValIleCysGluSerAlaGlyValGlnGluAspAlaAla
   GTTATCTGTGAAAGCGCGGGGTCCAGGAGGACGCGGCGAG
   CAATAGACACTTTTCGCGCCCCCAGGTCTCTCTGCGCGCTC

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**FIG. 30** Translation of DNA 26g

```

-----
1  GlyGlyGluAsnCysGlyTyrArgArgCysArgAlaSerGlyValLeuThrThrSerCys
   GGGGGGGAGAACTGCGGCTATCGCAGGTGCGCGCAAGGGGTACTGACAACCTAGCTGT
   CCCCCCTCTTGACGCCGATAGCGTCCACGGCGCGTTGCGCGCATGACTGTTGATCGACA
-----
61  GlyAsnThrLeuThrCysTyrIleLysAlaArgAlaAlaCysArgAlaAlaGlyLeuGln
   GGTAACACCTCCTCCTGTTTACATCAAGGCCCGAGCAGCCTGTGAGCCGCGAGGCTCCAG
   CCATTGTGGGAGTGAAATGTAGTTCCGGGCTGTCGGACAGCTCGGCGTCCCGAGGTC
-----
-----Overlap with 19g-----
121 AspCysThrMetLeuValCysGlyAspAspLeuValValIleCysGluSerAlaGlyVal
   GACTGCACCATGCTCGTGTGGCGACGACTTAGTCTTATCTGTGAAAGCGCGGGGTC
   CTGACGTGGTACGAGCACACACGCTGCTGAATCAGCAATAGACACTTTCGCGCCCCAG
-----
181 GlnGluAspAlaAlaSerLeuArgAlaPheThrGluAlaMetThrArgTyrSerAlaPro
   CAGGAGGACGCGCGGAGCCTGAGAGCCTTCACGGAGGCTATGACCAGGTACTCCGCCCCC
   GTCTCTCGCGCGCTCGGACTCTCGGAAGTGCTCGGATACTGGTCCATGAGGCGGGG
-----
241 ProGlyAspProProGlnProGluTyrAspLeuGluLeuIleThrSerCysSerSerAsn
   CCTGGGGACCCCCACAACCAAGATACGACTGGAGCTCATAACATCATGCTCTCCAAC
   GGACCCCTGGGGGGTGTGGTCTTATGCTGAACCTCGAGTATTGTAGTACGAGGAGGTTG
-----
301 ValSerValAlaHisAspGlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThr
   GTGTCACTGCGCCACGACGGCGCTGGAAGAGGGTCTACTACCTACCCGTCACCTTACA
   CACAGTCAGCGGGTCTGCGCGACCTTTCTCCAGATGATGGAGTGGGCACGAGGATGT
-----
361 ThrProLeuAlaArgAlaAlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeu
   ACCCCCCCTCGGAGAGCTGCGTGGGAGACAGCAAGACACACTCCAGTCAATTCTGGCTA
   TGGGGGGAGCGCTCTCGACGCAACCTCTGTCGTCTGTGTGAGGTCAGTTAAGGACCGAT
-----
421 GlyAsnIleIleMetPheAlaProThrLeuTrpAla
   GGCAACATAATCATGTTTGGCCCCACACTGTGGGCG
   CCGTGTATTAGTACAAACGGGGGTGTGACACCCGC

```

**FIG. 31** Translation of DNA 15e

```

-----
1  GlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAla
   CGGCGCTGGAAGAGGGTCTACTACCTACCCGTGACCTACAAACCCCTCGCGAGAGC
   GCGCGACCTTCTCCAGATGATGGAGTGGGCACTGGGATGTTGGGGGAGCGCTCTCG
-----
-----Overlap with 25g-----
61  AlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPhe
   TGGTGGGAGACAGCAAGACACACTCCAGTCAATTCTGCTAGGCAACATAATCATGTT
   ACGCACCTCTGTCTGTTGTGAGGTGAGTTAAGGACCGATCCGTTGTATTAGTACAA
-----
121 AlaProThrLeuTrpAlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAla
   TGCCCCCACTGTGGCGAGGATGATACTGATGACCCATTTCTTTAGCGTCTTATAGC
   ACGGGGGTGTGACACCGCTCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCG
-----
181 ArgAspGlnLeuGluGlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGlu
   CAGGACACAGCTTGAACAGGCCCTCGATGCGAGATCTACGGGGCTGCTACTCCATAGA
   GTCCCTGGTCAACTTGTCCGGGAGCTAACGCTCTAGATGCCCCGGACGATGAGGTATCT
-----
241 ProLeuAspLeuProProIleIleGlnArgLeu
   ACCACTTGATCTACCTCCAATCATTCAAAGACTC
   TGGTGAAGTAGATGGAGGTTAGTAAGTTCTGAG

```

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FIG. 32-1 COMBINED ORF OF DNAs 12f through 15e

IlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsn  
 1 CCATATTTAAATCAGGATGTACGTGGGAGGGGTGGAACACAGGCTGGAAGCTGCCTGCA  
 GGTATAAATTTTAGTCTACATGCACCTCCCCAGCTTGTGTCCGACCTTCGACGGAAGT

TrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeu  
 61 ACTGGACGCGGGCGAAGCTTGGATCTGGAAGACAGGGACAGGTCCGAGCTCAGCCCGT  
 TGACCTGCGCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGGCTCGAGTCGGGCA

LeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeu  
 121 TACTGTGACCACTACACAGTGGCAGTCTCCCGTGTCTTTCACAACTTACCAGCT  
 ATGACGACTGGTGATGTGTACCGTCCAGGAGGGCACAAGGAAGTGTGGGATGGTCGGA

SerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyVal  
 181 TGTCCACGGCTCATCCACCTCCACCAGAATCTGTGGAAGTGCAGTACTGTGAAGGG  
 ACAGGTGGCCGGAGTAGGTGGAGGTGGTCTTGTAAACCTGCAGCTCATGAACATGCCCC

GlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeu  
 241 TGGGGTCAAGCATCGCGTCTGGGCCATTAAGTGGGAGTACGTGTTCTCTCTGTCTCTTC  
 ACCCCAGTTCTGTAGCGCAGGACCGGTAATTCACCTCATGCAGCAAGAGGACAAGGAAG

LeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGlu  
 301 TGCTTGCAGACGCGCGCTCTGCTCTCTGCTTGTGGATGATGCTACTCATATCCCAAGCGG  
 ACGAAGCTCTGCGCGCGCAGACGAGGACCAACACCTACTACGATGAGTATAGGGTTCGCC

AlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeu  
 361 AGGCGGCTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGGCCGGGACGACCGGTC  
 TCCGCCGAAACCTCTTGGAGCATTATGAATTACGTCTGTAGGGACCGCCCTGCTGCCAG

ValSerPheLeuValPhePheCysPheAlaTrpTyrLeuLysGlyLysTrpValProGly  
 421 TTGTATCTTCTCTGTTCTTCTGCTTGTGATGGTATTGTGAAGGTAAAGTGGGTGCCCG  
 AACATAGGAAGGAGCACAAGAAGACGAAACGTACCATAAACTTCCATTACCCACGGGGC

AlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeuAlaLeuProGln  
 481 GAGCGGTCTACACCTTCTACGGGATGTGGCCTCTCTCTCTGCTCTGTGGCGTGGCCCC  
 CTCGCCAGATGTGGAAGATGCCCTACACGGAGAGGAGGACGAGGACAACCGCAACGGGG

ArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGlyValValLeuValGly  
 541 AGCGGGCGTACGCGCTGGACACGGAGGTGGCCGCGTCTGTGGCGGTGTGTCTCTGCTG  
 TCGCCGCTATGCGGACCTGTGCTCCACCGGCGCAGCACACCGCCACAACAAGAGCAGC

LeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSerTrpCysLeuTrpTrp  
 601 GGTGTATGGCGCTGACTCTGTACCATATTACAAGCGCTATATCAGCTGGTGTGTGGT  
 CCAACTACCGGACTGAGACAGTGGTATAATGTTCGCGATATAGTCGACCAAGAACCA

LeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrpIleProProLeuAsn  
 661 GGCTTCAGTATTTCTGACCAGAGTGGAGCGCACTGCAGTGTGGATTCCCCCTCA  
 CCGAAGTCATAAAGACTGGTCTACCTTCGCGTTGACGTGCACACCTAAGGGGGGAGT

ValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaValHisProThrLeuVal  
 721 ACGTCCGAGGGGGCGGACGCGCTCATCTTACTCATGTGTGCTGTACACCGACTCTGG  
 TGCAGGCTCCCCCGCGCTGCGGCAGTAGAATGAGTACACGACATGTGGGCTGAGACC

PheAspIleThrLysLeuLeuAlaValPheGlyProLeuTrpIleLeuGlnAlaSer  
 781 TATTTGACATCAACCAATGTCTGCTGGCGCTCTTCGGACCCCTTGGATTCTTCAAGCCA  
 ATAACTGTAGTGGTTAACGACGACCGGCAGAGCCTGGGGAACCTAAGAAGTTCGGT

LeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArgPheCysAlaLeuAla  
 841 GTTTGCTTAAAGTACCCTACTTTGTGCGCTCCAAGGCTTCTCCGGTCTTGCGGTTAG  
 CAAACGAATTTTCATGGATGAAACACGCGCAGGTTCGGGAAGAGCCAAGACGCGCAATC

ArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLysLeuGlyAlaLeuThr  
 901 CGCGGAAGATGATCGGAGGCCATTACGTGCAATGGTCATCATTAAAGTTAGGGGCGCTTA  
 GCGCTTCTACTAGCTCCGGTAATGCAGTTTACCAGTAGTAATTCATCCCCGCGAAT

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GlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAlaHisAsnGlyLeuArg  
 961 CTGGCACCTATGTTTATAACCATCTCACTCCTCTCGGGACTGGGCGCACAAACGGCTTC  
 GACCGTGGATACAAATATTGGTAGAGTGAGGAGAAGCCCTGACCGCGTGTTCGCGAACC  
 AspLeuAlaValAlaValGluProValValPheSerGlnMetGluThrLysLeuIleThr  
 1021 GAGATCTGGCCGTGGCTGTAGAGCCAGTCGTCTCTCCCAAATGGAGACCAAGCTCATCA  
 CTCTAGACCGGCACCGACATCTCGGTACAGCAGAAGAGGGTTTACCTCTGGTTCGAGTAGT  
 TrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeuProValSerAlaArg  
 1081 CGTGGGGGGCAGATACCGCCGCTGCGGTGACATCATCAACGGCTTGCTGTTCGCGCC  
 GCACCCCGCTCTATGGCGGCGCAGCCACTGTAGTAGTTGCCGAACGGACAAAGGCGGG  
 ArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSerLysGlyTrpArgLeu  
 1141 GCAGGGGCGGGAGATAGTGTCTGGGCCAGCCGATGGAATGGTCTCCAAGGGGTGGAGGT  
 CGTCCCGGCCCTCTATGACGAGCCCGGTGCGCTACCTTACCAGAGGTTCACCACTCCA  
 LeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeuGlyCysIleIleThr  
 1201 TGCTGGCGCCATCACGGGTACGCCAGCAGACAAGGGGCTCTAGGGTGCAATATCA  
 ACGACCGCGGGTAGTGCCGATGCGGTGCTGTGTCCCGGAGGATCCACGTATTAGT  
 SerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGlnIleValSerThrAla  
 1261 CCAGCCTAAGTGGCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAGATGTGTCAACTG  
 GGTGGATTGACCGGCCCTGTTTTTGGTTACCTCCACTCCAGGTCTAACACAGTTGAC  
 AlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThrValTyrHisGlyAla  
 1321 CTGCCCCAAACCTTCCTGGCAACGTGTCATCAATGGGGTGTGCTGGACTGTCTACCAACGGGG  
 GACGGGTTTGAAGGACCGTTGCACGTAGTTCACCCACAGACCTGACAGATGGTGCCCC  
 GlyThrArgThrIleAlaSerProLysGlyProValIleGlnMetTyrThrAsnValAsp  
 1381 CCGGAACGAGGACCATGCGCTACCCCAAGGGTCCCTGTCTATCCAGATGTATACCAATGTAG  
 GGCCCTGTCTCTGGTAGCGCAGTGGGTTCACGAGCAGTAGGTCTACATATGGTTACATC  
 GlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeuThrProCysThrCys  
 1441 ACCAAGACCTTGTGGGTGGCCCGCTCCGCAAGGTAGCGCTCATTGACACCCCTGCACCTT  
 TGGTTCTGGAACACCCGACCGGGCGAGGCGTTCCATCGCGGAGTAACGTGGGACGTGAA  
 GlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIleProValArgArgArg  
 1501 GCGGCTCCTCGGACCTTACCTGGTCAAGGACAGGCGCGATGTCTATCCCGTGGCGCGGC  
 CGCCGAGGAGCCTGGAATGGACAGTGTCTCCGTGCGGTACAGTAAGGGCAGCGGGCGG  
 GlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyrLeuLysGlySerSer  
 1561 GGGGTGATAGCAGGGGCGGCTGTGTGCGCCCGGCCATTCTCTACTGAAAGGCTCCT  
 CCCCCTATCTCTCCCGTGGACGACAGCGGGGCGGGTAAAGGATGAACCTTCCGAGGA  
 GlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePheArgAlaAlaValCys  
 1621 CGGGGGTCCGCTGTGTGTCGCCCGGGGCAAGCGTGGGCATATTTAGGGCGCGGTGT  
 GCCCCCAGGOGACAACACGGGGCGCCCGTGGCGCACCGTATAAATCCGGCGCCACA  
 ThrArgGlyValAlaLysAlaValAspPheIleProValGluAsnLeuGluThrThrMet  
 1681 GCAACCGTGGAGTGGCTAAGGCGGTGACTTTATCCCTGTGGAGAACCTAGAGACAACCA  
 CGTGGGCACCTACCGATTCCGCCACTGAAATAGGGACACCTCTGGATCTCTGTGGT  
 ArgSerProValPheThrAspAsnSerSerProProValValProGlnSerPheGlnVal  
 1741 TGAGGTCCCGGTGTTACCGATAACTCCTCTCCACAGTAGTGCCCGAGAGCTTCCAGG  
 ACTCCAGGGGCCACAAGTGCTATTGAGGAGAGGTGGTTCATCAGGGGTCTCGAAGGTCC  
 AlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysValProAlaAlaTyrAla  
 1801 TGGCTCACCTCCATGCTCCACAGGCGGGCAAAAGCAACCAAGGTCCCGGCTGCATATG  
 ACCGAGTGGAGGTACGAGGTGTCGCTCGCGTPTTGTGGTTCCAGGCGCAGCTATAC  
 AlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAlaThrLeuGlyPheGly  
 1861 CAGCTCAGGGCTATAAGGTGCTAGTACTCAACCCCTCTGTGTGTCGAACACTGGGCTTTG  
 GTCGAGTCCCGATATCCACGATCATGAGTTGGGAGACAACGACGTTGTGACCCGAAAC  
 AlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThrGlyValArgThrIle

FIG. 32-2

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1921 GTGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACGGGGTGAGAACA  
CACGAATGTACAGGTTCCGAGTAOCCTAGCTAGGATTGTAGTCTGGCCCCACTCTTGT

ThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCys

1981 TTACCACTGGCAGCCCATCAGTACTCCACCTAOGGCAAGTTCCTTGCCGACGGGGGT  
AATGGTGACCGTGGGGTAGTGATGAGGTGGATGCCGTTCAAGGAAGGCTGCCGCCA

SerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSerThrAspAlaThrSer

2041 GCTCGGGGGGCGCTTATGACATAATAATTGTGAOGAGTGCCACTCCACGGATGCCACAT  
CGAGCCCCCGCAATACTGTATTATTAAACACTGCTCAGGTGAGGTGCCTACGGTGTAT

IleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValVal

2101 CCATCTTGGGCATCGGCACTGTCTTGACCAAGCAGAGACTGCGGGGGCGAGACTGGTTG  
GGTAGAACCGTAGCGTGACAGGAACGTGTTCTCTGACGCCCCCGCTCTGACCAAC

LeuAlaThrAlaThrProProGlySerValThrValProHisProAsnIleGluGluVal

2161 TGCTCGCCACCGCCACCCCTCCGGGCTCCGTCCTGTGCCCCATCCCAACATCGAGGAGG  
ACGAGCGGTGGCGGTGGGAGGCCCGAGGCAGTGACCGGGTAGGGTGTAGCTCCTCC

AlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIleProLeuGluValIle

2221 TTGCTCTGTCCACCACCGGAGAGATCCCTTTTTCAGGCAAGGCTATCCCCCTCGAAGTAA  
ACGAGACAGGTGGTGGCTCTCTAGGAAATGCGTTCCGATAGGGGAGCTTCATT

LysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCysAspGluLeuAlaAla

2281 TCAAGGGGGGAGACATCTCATCTTCTGTCTTCAAGAAGAAGTGCGACGAAGTCCGCG  
AGTTCCCCCTCTGTAGAGTAGAAGACAGTAAGTTTCTTCTCACGCTGCTTGAGCGC

LysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerVal

2341 CAAAGCTGGTGCATTTGGGCATCAATGCCGTGGCTACTACCGCGGTCTTGAGTGTCCG  
GTTTCCAGCGGTAAACCGTAGTTACGGCACCGGATGATGGGCCAGAACTGCACAGGC

IleProThrSerGlyAspValValValAlaThrAspAlaLeuMetThrGlyTyrThr

2401 TCATCCGACCGAGCGGATGTGTCTGTGGCAACCGATGCCCTCATGACCGGCTATA  
AGTAGGCTGGTCCGCTACAAACAGCAGCACCGTTGGCTAGGGAGTACTGGCGATAT

GlyAspPheAspSerValIleAspCysAsnThrCysValThrGlnThrValAspPheSer

2461 CCGGCGACTTCGACTCGGTGATAGACTGCAATACGTGTGTCAACAGACAGTGGATTCA  
GGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGTCTGTACGCTAAAGT

LeuAspProThrPheThrIleGluThrIleThrLeuProGlnAspAlaValSerArgThr

2521 GCCTTGACCTACCTTCACCATGAGACAATCAGCTCCCCAGGATGCTGTCTCCCGCA  
CGGAACCTGGGATGGAAGTGGTAACCTGTGTAGTGGAGGGGTCTACGACAGAGGGGT

GlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArgPheValAlaProGly

2581 CTCAACGTCCGGGCGAGTGGCAGGGGGAAGCCAGGCATCTACAGATTGTGGCACCGG  
GAGTTGCAGCCCGTCTGACCGTCCCCCTCGGTCCGTAGATGTCTAAACACCGTGGCC

GluArgProSerGlyMetPheAspSerSerValLeuCysGluCysTyrAspAlaGlyCys

2641 GGGAGCGCCCCCTCCGGCATGTTGACTCGTCCGTCTCTGTGAGTGTATGACGAGGCT  
CCCTCGCGGGAGGCCGTACAAGCTGAGCAGGCAGGAGACACTACGATACTGCGTCCGA

AlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThr

2701 GTGCTTGGTATGAGCTCAGCCCGCGAGACTACAGTTAGGCTACGAGCGTACATGAACA  
CACGAACCATACTCGAGTGGGGCGGCTCTGATGTCAATCGATGCTCGCATGTACTGT

ProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeu

2761 CCCCGGGGCTCCCGTGTGCCAGGACCATCTTGAATTTGGGAGGGCGTCTTACAGGCC  
GGGGCCCCGAAGGCGACACGGTCTGTAGAACTTAAACCTCCCGCAGAAATGTCCGG

ThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyr

2821 TCACTCATATAGATGCCACTTTCTATCCAGACAAAGCAGAGTGGGAGAACTTCCTT  
AGTGATATATCTACGGGTGAAGATAGGGTCTGTTTGTCTCACCCCTCTTGGAAAGAA

LeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAsp

2881 AOCCTGGTAGCTACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCCTCCCATCGTGGG  
TGGACCATCGCATGGTCCGTGGCACACGCGATCCCGAGTTGGGGAGGGGTAGCACCC

FIG. 32-3

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GlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeu  
 2941 ACCAGATGTGGAAGTGTTTGATTGGCTCAAGCCACCTCCATGGCCCAACCCCTGC  
 TGGTCTACACCTTCACAACTAAGCGGAGTTGGGTGGGAGGTACCCGGTGTGGGGAGC

TyrArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIle  
 3001 TATACAGACTGGGCGCTGTTCAGAAATGAAATCACCTTACGACCCAGTCACCAAATACA  
 ATATGTCTGACCCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGGTGAGTGGTATTATGT

MetThrCysMetSerAlaAspLeuGluValValThrSerThrTrpValLeuValGlyGly  
 3061 TCATGACATGTCATGTGGGCGACCTGGAGGTGTCAAGACACCTGGGTGCTCGTTGGCG  
 AGTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTGTGTGTGGACCCACGAGCAACCGC

ValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArg  
 3121 GCGTCTGGCTGCTTTGGGCGCGTATTGCTGTCAACAGGCTGCGTGGTCAAGTGGGCA  
 CGCAGGACCGACGAAACCGGCGCATACGAGACAGTTGTCCGACGCCAGTATCACCCGT

ValValLeuSerGlyLysProAlaIleIleProAspArgGluValLeuTyrArgGluPhe  
 3181 GCGTCGTCTGTTCGGAAGCCGCAATCATACCTGACAGGGAAGTCTCTACCGAGAGT  
 CCCAGCAGAACAGGCCCTTCGGCGCTTAGTATGGACTGTCCCTTCAGGAGATGGCTCTCA

AspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAla  
 3241 TCGATGAGATGGAAGAGTGTCTTCAGCACTTACGTACATCGAGCAAGGGATGATGCTCG  
 AGCTACTCTACCTTCTCAGAGAGTGTGTAATGGCATGTAGCTCGTTCCCTACTAOGAGC

GluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluVal  
 3301 CCGAGCAGTTCAAGCAGAAGCCCTCGGCTCCTGCAGACCGCGTCCCGTCAGGCAGAGG  
 GGCTGTCAAGTTCGTCTTCGGGAGCCGAGGACGTCTGGCGCAGGGCAGTCCGTCTCC

IleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMet  
 3361 TTATGGCCCTGTGTTCAGACCACTGGCAAACTCGAGACCTTCTGGGCGAAGCATA  
 AATAGCGGGACGACAGGTCTGGTTGACCGTTTTTGAGCTCTGGAAGACCCGCTCGTAT

TrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnPro  
 3421 TGTGGAACCTTCATCAGTGGGATACAATACTTGGCGGGCTTGTCAACGCTGCTGGTAACC  
 ACACCTTGAAGTAGTACCCCTATGTTATGAACGCGCCGAACAGTTGCGACGACCATTTGG

AlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGln  
 3481 CCGCCATTGCTTCATGTATGGCTTTTACAGCTGCTGTCAACGACCCACTAACCCTAGCC  
 GGGCGTAACGAAGTAACCTACGAAATGTGACGACAGTGTGCGGTGATTGCTGATCGG

ThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeuAlaAlaProGlyAla  
 3541 AAACCTCTCTTCAACATATGGGGGGTGGGTGGCTGCCAGCTGCCGCCCCCGGTG  
 TTTGGGAGGAGAAGTTGTATAACCCCCCACCACCGACGGTTCGAGCGGCGGGGGCCAC

AlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGlySerValGlyLeuGly  
 3601 CCGCTACTGCTTTGTGGGCGCTGGCTTAGCTGGGCGCGCATCGGCAGTGTGGACTGG  
 GCGATGACGGAACACCCGCGACCGAATCGACCGCGCGGTAGCCGTCACAACCTGACC

LysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAlaGlyAlaLeuValAla  
 3661 GGAAGGTCTCATAGACATCTTGCAGGGTATGGCGGGGCTGGCGGGAGCTCTTGTGG  
 CCTCCAGGAGTATCTGTAGGAACGTCCCATACCGCGCCGACCGCCCTCGAGAACACC

PheLysIleMetSerGlyGluValProSerThrGluAspLeuValAsnLeuLeuProAla  
 3721 CATTCAGATCATGAGCGGTGAGTCCCTCCACGAGGACCTGGTCAATCTACTGCCCG  
 GTAGTTCTAGTACTGCCACTCCAGGGAGGTGCTCTCTGGACAGTTAGATGACGGGC

IleLeuSerProGlyAlaLeuValValGlyValValCysAlaAlaIleLeuArgArgHis  
 3781 CCATCCTCTCGCCCGAGCCCTGTAGTGGGCTGGTCTGTGCAGCAATACTGGCGCGGC  
 GGTAGGAGAGCGGGCTCGGGAGCATCAGCGCACAGACAGTCTGTATGACGCGGGCG

ValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIleAlaPheAlaSerArg  
 3841 ACCGTGGCCCGGGCGAGGGGCGAGTGCAGTGGATGAACGGCTGATAGCCTTCGCTCCC  
 TGCAACGGGCGCGCTCCCGCTCACTTACCTTCTGGCGACTATCGGAAGCGGAGGG

GlyAsnHisValSerProThrHisTyrValProGluSerAspAlaAlaAlaArgValThr

FIG. 32-4

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3901 GGGGGAACCATGTTTCCCCACGCACTAAGTCCGAGAGCGATGCAGCTGCCCGCGTCA  
 CCCCCTGGTACAAAGGGGGTGGTGATGCAAGGCTCTCGCTACGTGACGGGCGCAGT

AlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeuHisGlnTrpIleSer  
 3961 CTGCCATACTCAGCAGCTCACTGTAAACCAGCTCTGAGGCGACTGCACCAGTGGATAA  
 GACGGTATGAGTGTGAGTGACATTGGGTGAGGACTCCGCTGACGTGGTCACCTATT

SerGluCysThrThrProCysSerGlySerTrpLeuArgAspIleTrpAspTrpIleCys  
 4021 GCTCGGAGTGTACCACTCCATGCTCCGGTCTCTGGCTAAGGGACATCTGGGACTGGATAT  
 CGAGCCTCACATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAGACCTGACCTATA

GluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMetProGlnLeuProGly  
 4081 GCGAGGTGTTGAGCGACTTTAAGACCTGGCTAAAAGCTAAGCTCATGOCACAGCTGCCTG  
 CGCTCCACAACCTCGCTGAAATCTGGAACGATTTCGATTGAGTACGGTGTGACGGAC

IleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArgValAspGlyIleMet  
 4141 GGATCCCTTTGTGTCTGCCAGCGCGGTATAAGGGGCTCTGGGAGTGGACGGCATCA  
 CCTAGGGGAACACAGGAAGGTGCGCCCATATTCCCCAGACCGCTACCTGCGGTAGT

HisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLysAsnGlyThrMetArg  
 4201 TGCACACTCGCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAAAACGGGACGATGA  
 ACGTGTGAGCGACGGTGACACCTGACTCTAGTGACCTGTACAGTTTGTGCCCTGCTACT

IleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPheProIleAsnAlaTyr  
 4261 GGATCGTGGGTCTTAGGACCTGCAGGAACATGTGGAGTGGGACCTTCCCATTAATGCT  
 CCTAGCAGCCAGGATCTGGACGCTCTGTACACCTCAOCTGGAAGGGGTAATTACGGA

ThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPheAlaLeuTrpArgVal  
 4321 ACACCACGGGCCCTGTACCCCTTCCTGCGCGAATACAGCTTGGCGCTATGGAGGG  
 TGTGGTGGCCGGGACATGGGGGAAGGACGGGCTTGATGTGCAAGCGGATACCTCC

SerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHisTyrValThrGlyMet  
 4381 TGTCTGCAGAGGAATATGTGGAGATAAGGCAAGTGGGGGACTTCCACTACGTGACGGGTA  
 ACAGAGCTCTCTTATACACCTCTATTCCGTCCACCCCTGAAAGGTGATGCAGTCCCAT

ThrThrAspAsnLeuLysCysProCysGlnValProSerProGluPhePheThrGluLeu  
 4441 TGACTACTGACAATCTCAATGCCCCGTGCCAGGTCCCATCGCCCGAATTTTTCACAGAAT  
 ACTGATGACTGTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTTAAAAAGTGCTTA

AspGlyValArgLeuHisArgPheAlaProProCysLysProLeuLeuArgGluGluVal  
 4501 TGAACGGGGTGGCCCTACATAGGTTTGGCCCCCTGCAGCCCTTCTGCGGGAGGAGG  
 ACCTGCCCCACGGGATGTATCCAAAGCGGGGGACGTTGGGAACGACGCCCTCTCC

SerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeuProCysGluProGlu  
 4561 TATCATTCAGAGTAGGACTCCACGAATACCCGGTAGGGTCGCAATTACCTTGGAGCCCG  
 ATAGTAAGTCTCATCTGAGGTGCTTATGGGCCATCCAGCGTTAATGGAACGCTCGGGC

ProAspValAlaValLeuThrSerMetLeuThrAspProSerHisIleThrAlaGluAla  
 4621 AACCGGACGTGGCCGTGTTGACGTCCATGCTCACTGATCCCTCCCATATAACAGCAGAGG  
 TTGGCCTGACCGGCACAACCTGCAGGTACGAGTGACTAGGGAGGGTATATTGTCGTCTCC

AlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSerSerSerAlaSerGln  
 4681 CGGCCGGCGAAGGTTGGCGAGGGGATCACCCTCTGTGGCCAGCTCTCGGCTAGCC  
 GCGGCCCGCTTCCAAACCGCTCCCTAGTGGGGGAGACACGGTTCGAGGAGCCGATCGG

LeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAspSerProAspAlaGlu  
 4741 AGCTATCCGCTCCATCTCTCAAGGCAACTTGCACCGCTAACCATGACTCCCTGATGCTG  
 TCGATAGGCGAGGTAGAGATTCCGTTGAACGTGGCGATTGGTACTGAGGGGACTACGAC

LeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsnIleThrArgValGlu  
 4801 AGCTCATAGAGCCCAACCTCTATGGAGGAGGATGGGCGGCAACATCACCAGGCTTG  
 TCGAGTATCTCGGTGGAGGATACCTCGTCTCTACCGCGTTGTAGTGGTCCCAAC

SerGluAsnLysValValIleLeuAspSerPheAspProLeuValAlaGluGluAspGlu  
 4861 AGTCAGAAAACAAAGTGGTATTCTGGACTCCTTCGATCCGCTTGTGGCGGAGGAGGACG  
 TCAGTCTTTTGTTCACCACTAAGACCTGAGGAAGCTAGGCGAACACCGCTCTCTCTGC

FIG. 32-5

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ArgGluIleSerValProAlaGluIleLeuArgLysSerArgArgPheAlaGlnAlaLeu  
 4921 AGCGGGAGATCTCCGTACCGCAGAAATCCTGCGGAAGTCTCGGAGATTGCCCCAGGCC  
 TCGCCCTCTAGAGGCATGGGCGTCTTTAGGACGCCCTCAGAGCCTTAAGCGGGTCCGGG  
  
 ProValTrpAlaArgProAspTyrAsnProProLeuValGluThrTrpLysLysProAsp  
 4981 TGCCCGTTTGGGCGCGGCGGACTATAACCCCCGCTAGTGAGACGTGGAAAAAGCCCG  
 ACGGGCAAACCGCGCGCGCTGATATTGGGGGGGATCACCTCTGCACCTTTTCGGGC  
  
 TyrGluProProValValHisGlyCysProLeuProProProLysSerProProValPro  
 5041 ACTACGAACCACTGTGGTCCATGGCTGTCCGCTTCCACCTCCAAAGTCCCTCCTGTGC  
 TGATGCTTGGTGACACCCAGGTACGACAGGCGAAGGTGGAGGTTTCAGGGGAGGACAG  
  
 ProProArgLysLysArgThrValValLeuThrGluSerThrLeuSerThrAlaLeuAla  
 5101 CTCGCGCTCGGAAGAAGCGGACGGTGTCTCACTGAATCAACCTATCTACTGCCTTGG  
 GAGGCGGAGCCTTCTTCGCTGCCACCAGGAGTGACTTAGTTGGGATAGATGACGGAACC  
  
 GluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIleThrGlyAspAsnThr  
 5161 CCGAGCTCGCCACCAGAAGCTTTGGCAGCTCCTCAACTTCGCGATTACGGGCGACAATA  
 GGCTCGAGCGGTGGTCTTCGAAACCGTCGAGGAGTTGAAGGCCGTAATGCCCGCTGTAT  
  
 ThrThrSerSerGluProAlaProSerGlyCysProProAspSerAspAlaGluSerTyr  
 5221 CGACAACATCCTCTGAGCCCGCCCCCTCTGGCTGCCCCCGGACTCGACGCTGAGTCCT  
 GCTGTTGTAGGAGACTCGGGCGGGGAAGACCGACGGGGGGGCTGAGGCTGCGACTCAGGA  
  
 SerSerMetProProLeuGluGlyGluProGlyAspProAspLeuSerAspGlySerTrp  
 5281 ATTCTCCATGCCCCCTCGGAGGGGAGCCTGGGGATCCGATCTTAGCGAGCGGTCAT  
 TAGGAGGTACGGGGGGGACCTCCCCCTCGGACCCCTAGGCCTAGAATCGTCCCGCAT  
  
 SerThrValSerSerGluAlaAsnAlaGluAspValValCysCysSerMetSerTyrSer  
 5341 GGTCAACGGTCAGTAGTGAGGCCAACCGGAGGATGTGCTGTGCTGCTCAATGTCTTACT  
 CCAGTTGCCAGTCATCACTCGGTTGCGCCTCTACAGCACACGAGGTTACAGAATGA  
  
 TrpThrGlyAlaLeuValThrProCysAlaAlaGluGluGlnLysLeuProIleAsnAla  
 5401 CTGGACAGGCGCACTCGTCACCCCGTGGCGCGCGGAAGAACAGAACTGCCCATCAATG  
 GAACCTGTCCGCTGAGCAGTGGGCGACGCGCGCCTCTTGTCTTTGACGGGTAGTTAC  
  
 LeuSerAsnSerLeuLeuArgHisHisAsnLeuValTyrSerThrThrSerArgSerAla  
 5461 CACTAAGCAACTCGTTGCTACGTCACCACAATTGGGTATTCACCACCTCAGCGAGTG  
 GTGATTCTGTTAGCAACGATGCAGTGGTGTAAACCATTAAGGTGGTGGAGTGCCTCAC  
  
 CysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeuAspSerHisTyrGln  
 5521 CTTGCCAAAGGCAGAAAGTACATTGTACAGACTCAAGTTCTGGACAGCCATTACC  
 GAACGGTTTCGCTCTCTTTCAGTGTAACCTGTCTGAGCTCAAGACCTGTGGGTAAATGG  
  
 AspValLeuLysGluValLysAlaAlaAlaSerLysValLysAlaAsnLeuLeuSerVal  
 5581 AGGACGTACTCAAGGAGGTTAAAGCAGCGGCGTCAAAAGTGAAGGCTAACTGTCTATCCG  
 TCCTGCATGAGTTCTCTCAATTTCTGTCGCGCAGTTTTCACCTTCCGATTGAACGATAGGC  
  
 GluGluAlaCysSerLeuThrProProHisSerAlaLysSerLysPheGlyTyrGlyAla  
 5641 TAGAGGAAGCTTGCAGCTGACGCCCCCACTCAGCCAAATCCAAGTTTGGTTATGGGG  
 ATCTCCTTCGAACGTGCGACTGCGGGGGTGTGAGTGGTTTAGGTTCAAACCAATACCCC  
  
 LysAspValArgCysHisAlaArgLysAlaValThrHisIleAsnSerValTrpLysAsp  
 5701 CAAAAGACGTCCGTTGCCATGCCAGAAAGGCGTAACCCACATCACTCCGTGTGGAAAG  
 GTTTTCTGCAGGCAACGGTACGGTCTTTCGGCATTGGGTGTAGTTGAGGCACACCTTTC  
  
 LeuLeuGluAspAsnValThrProIleAspThrThrIleMetAlaLysAsnGluValPhe  
 5761 ACCTTCTGGAAGACAATGTACACCAATAGACACTACCATCATGGCTAAGAAGAGGTTT  
 TGAAGACCTTCTGTTACATTGTGGTTATCTGTGATGGTAGTACCGATTCTTGCTCCAAA  
  
 CysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIleValPheProAspLeu  
 5821 TCTCGGTTTCAGCCTGAGAAGGGGGTCTGAAGCCAGCTCGTCTCATCGTGTTCCTCGATC  
 AGACGCAAGTCCGACTCTTCCCCCAGCATTOGGTGCAGCAGAGTAGCACAGGGGCTAG  
  
 GlyValArgValCysGluLysMetAlaLeuTyrAspValValThrLysLeuProLeuAla

FIG. 32-6

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5881 TGGGCGTGGCGGTGTGCGAAAAGATGGCTTTGTACGACGTGGTTACAAAGCTCCCTTGG  
ACCCGACGCGCACACGCTTTTCTACCGAAACATGCTGCACCAATGTTTCGAGGGGAACC

ValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArgValGluPheLeuVal  
5941 CCGTGATGGGAAGCTCCTACGATTCCTCAATACTCACCAGGACAGCGGGTTGAATTCCCTCG  
GGCACTACCTTCGAGGATGCTAAGGTTATGAGTGGTCTGTGCGCCAACTTAAGGAGC

GlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAspThrArgCysPheAsp  
6001 TGCAAGCGTGGGAAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGATACCGCTGCTTTG  
ACGTTGCGACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTATGGGCGACGAAAC

SerThrValThrGluSerAspIleArgThrGluGluAlaIleTyrGlnCysCysAspLeu  
6061 ACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGGCAATCTACCAATGTTGTGACC  
TGAGGTGTCAGTGACTCTCGCTGTAGGCATGCCTCCTCGTTAGATGGTTACAACACTGG

AspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeuTyrValGlyGlyPro  
6121 TCGACCCCAAGCCCGCGTGGCCATCAAGTCCCTCACCGAGAGGCTTTATGTTGGGGGCC  
AGCTGGGGGTTCCGGCGCACCGGTAGTTCAGGGAGTGGCTCTCGAAATACAACCCCGG

LeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArgAlaSerGlyValLeu  
6181 CTCTTACCAATTCAAGGGGGGAGAACTGCGGCTATCGCAGGTGCCGCGGAGCGGGCTAC  
GAGAAATGGTTAAGTTCCCCCTCTTGACGCCGATAGCGTCCACGGCGGCTCGCGCGATG

ThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArgAlaAlaCysArgAla  
6241 TGACAACTAGCTGTGGTAACACCCTCACTTGCTACATCAAGCCCCGGGCGAGCTGTGAG  
ACTGTTGATCGACACCATTGTGGGAGTGAAAGTAGTTCGGGGCCCGTGGACAGCTC

AlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeuValValIleCysGlu  
6301 CCGCAGGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTAGTCGTTATCTGTG  
GGCGTCCGAGGTCTGACGTGGTACGAGCACACCGCTGTGTAATCAGCAATAGACAC

SerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThrGluAlaMetThrArg  
6361 AAAGCGCGGGGGTCCAGGAGGACGCGCGGAGCCTGAGAGCCTTCACGGAGGCTATGACCA  
TTTCGCGCCCCCAGGTCTCTCGCGCGCTCGGACTCTCGGAAGTGCTCCGATACTGGT

TyrSerAlaProProGlyAspProProGlnProGluTyrAspLeuGluLeuIleThrSer  
6421 GGTACTCCGCCCCCTGGGGACCCCCACAACCAAGAAATACGACTTGGAGCTCATAACAT  
CCATGAGGCGGGGGGACCCCTGGGGGGTGTGGTCTTATGCTGAACCTGAGTATTGTA

CysSerSerAsnValSerValAlaHisAspGlyAlaGlyLysArgValTyrTyrLeuThr  
6481 CATGCTCCTCCAACGTGTCTAGTGGCCACGACGGGCTGGAAAGAGGGTCTACTACCTCA  
GTACGAGGAGGTGACAGTCAGCGGGTGTGCGCGACCTTCTCCAGATGATGAGT

ArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAlaArgHisThrProVal  
6541 CCGTGACCCCTACAACCCCTCGCGAGAGCTGGTGGGAGACAGCAAGACACACTCCAG  
GGGCACTGGGATGTTGGGGGGAGCGCTCTCGACGACCCCTGTCTGTTCTGTGTGAGGTC

AsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrpAlaArgMetIleLeu  
6601 TCAATTCTGGCTAGGCAACATAATCATGTTTGGCCCCACACTGTGGGOGAGGATGATAC  
AGTTAAGGACCGATCCGTTGTATTAGTACAAACGGGGGTGTGACACCGCTCCTACTATG

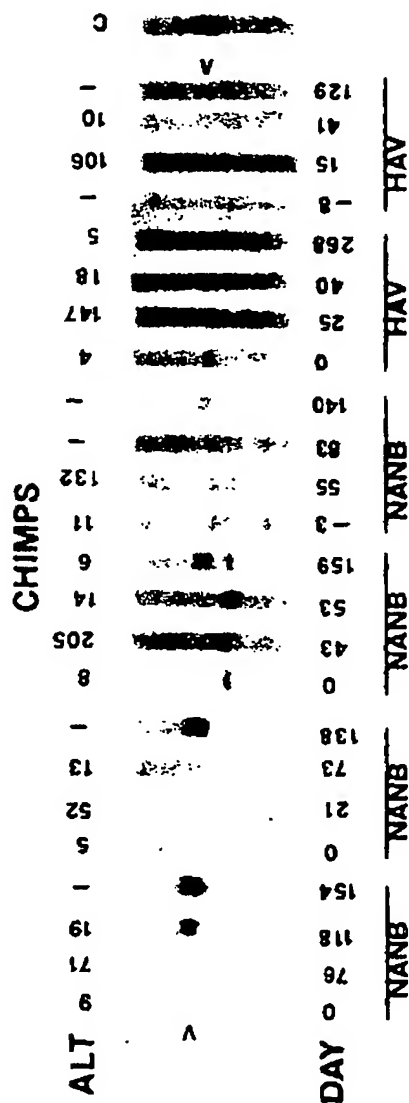
MetThrHisPhePheSerValLeuIleAlaArgAspGlnLeuGluGlnAlaLeuAspCys  
6661 TGATGACCAATTCTTTAGCGTCCTTAGCCAGGGACGCTTGAACAGGCCCTCGATT  
ACTACTGGGTAAAGAAATCGCAGGAATATCGGTCCCTGGTGAACCTTGTCGGGAGCTAA

GluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuProProIleIleGlnArg  
6721 GCGAGATCTACGGGCTGTCTACTCCATAGAACCACTTGATCTACCTCCAATCATTCAAA  
CGCTCTAGATGCCCCGACGATGAGGTATCTTGGTGAACCTAGATGGAGGTTAGTAAGTTT

Leu  
6781 GACTC  
CTGAG

FIG. 32-7

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**FIG. 33** LEGEND

Lane Number	Chimp Reference Number	Infection Type	Sample date (days) (0=inoculation day)	ALT (alanine) aminotransferase level in sera) $\mu\text{U/ml}$
1	1	NANB	0	9
2	1	NANB	76	71
3	1	NANB	118	19
4	1	NANB	154	N/A
5	2	NANB	0	5
6	2	NANB	21	52
7	2	NANB	73	13
8	2	NANB	138	N/A
9	3	NANB	0	8
10	3	NANB	43	205
11	3	NANB	53	14
12	3	NANB	159	6
13	4	NANB	-3	11
14	4	NANB	55	132
15	4	NANB	83	N/A
16	4	NANB	140	N/A
17	5	HAV	0	4
18	5	HAV	25	147
19	5	HAV	40	18
20	5	HAV	268	5
21	6	HAV	-8	N/A
22	6	HAV	15	106
23	6	HAV	41	10
24	6	HAV	129	N/A
26	7	HAV	0	7
27	7	HAV	22	83
28	7	HAV	115	5
29	7	HAV	139	N/A
30	8	HAV	0	15
31	8	HAV	26	110
32	8	HAV	74	8
33	8	HAV	205	5
34	9	HBV	-290	N/A
35	9	HBV	379	9
36	9	HBV	435	6
37	10	HBV	0	8
38	10	HBV	111-118 (pool)	96-156 (pool)
39	10	HBV	205	9
40	10	HBV	240	13
41	11	HBV	0	11
42	11	HBV	28-56 (pool)	8-100 (pool)
43	11	HBV	169	9
44	11	HBV	223	10

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**FIG. 34** LEGEND

Lane Number	Patient Reference Number	Diagnosis	ALT Level (mu/ml)
1	1	NANB	1354
2	1 <sup>1</sup>	NANB	31
3	2 <sup>1</sup>	NANB	14
4	2 <sup>2</sup>	NANB	79
5	2 <sup>3</sup>	NANB	26
6	3 <sup>1</sup>	NANB	78
7	3 <sup>2</sup>	NANB	87
8	3 <sup>3</sup>	NANB	25
9	4 <sup>1</sup>	NANB	60
10	4 <sup>2</sup>	NANB	13
11	5 <sup>1</sup>	NANB	298
12	5 <sup>2</sup>	NANB	101
13	6 <sup>1</sup>	NANB	474
14	6 <sup>2</sup>	NANB	318
15	7 <sup>1</sup>	NANB	20
16	7 <sup>2</sup>	NANB	163
17	8 <sup>1</sup>	NANB	44
18	8 <sup>2</sup>	NANB	50
19	9	NANB	N/A
20	10	NANB	N/A
21	11	NANB	N/A
22	12	Normal	N/A
23	13	Normal	N/A
24	14	Normal	N/A
26	30174	Normal	N/A
27	30105	Normal	N/A
28	30072	Normal	N/A
29	30026	Normal	N/A
30	30146	Normal	N/A
31	30250	Normal	N/A
32	30071	Normal	N/A
33	15	AcuteHAV	N/A
34	16	AcuteHAV	N/A
35	17	AcuteHAV	N/A
36	18	AcuteHAV	N/A
37	48088	AcuteHAV	N/A
38	47288	AcuteHAV	N/A
39	47050	AcuteHAV	N/A
40	46997	AcuteHAV	N/A
41	19	Convalescent HBV	N/A
42	20	{anti-HBSag+ve;	N/A
43	21	{anti-HBCag+ve}	N/A
44	22	{anti-HBSag+ve;	N/A
45	23	{anti-HBCag+ve}	N/A
46	24	{anti-HBSag+ve;	N/A
47	25	{anti-HBCag+ve}	N/A
48	26	{anti-HBSag+ve;	N/A
49	27	{anti-HBSag+ve}	N/A

<sup>1</sup> Sequential serum samples were assayed from these patients

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FIG. 34-1

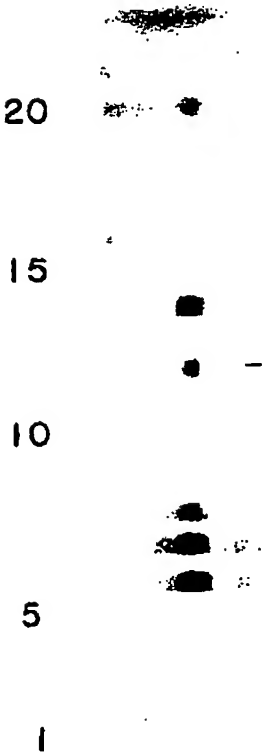
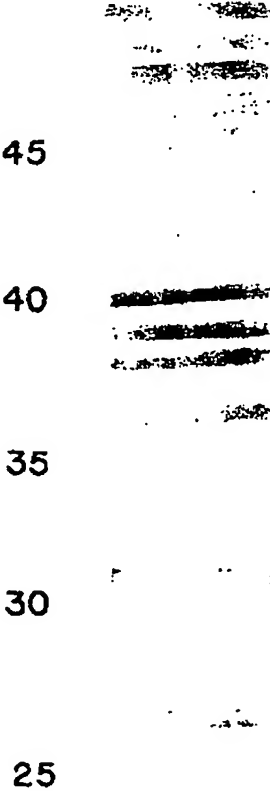
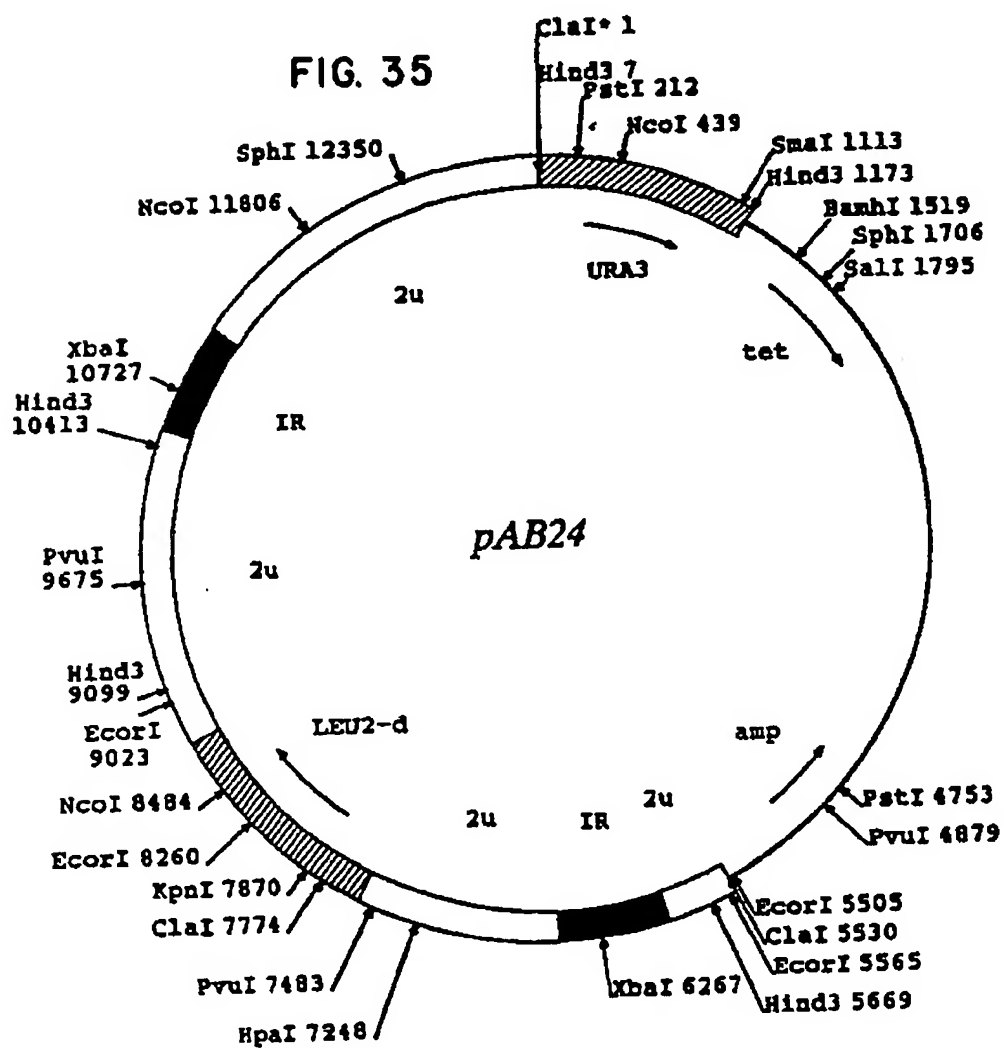


FIG. 34-2



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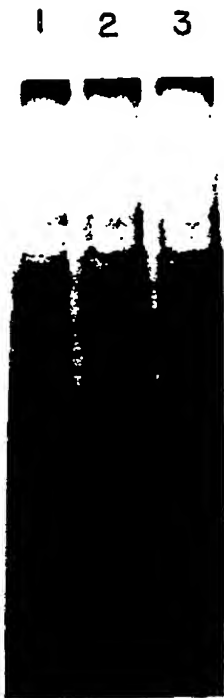


FIG. 37 a



FIG. 37 b

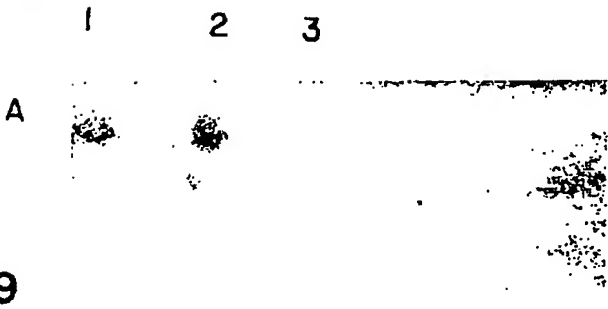


FIG. 39

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FIG. 38

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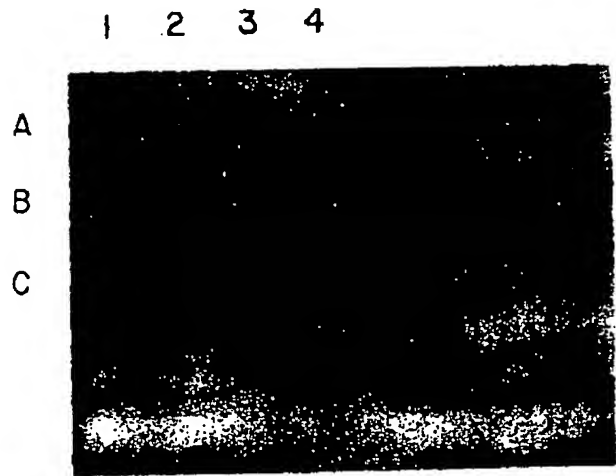


FIG. 40

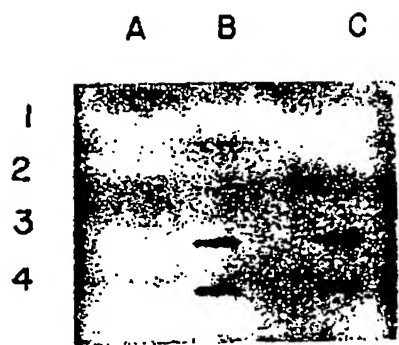


FIG. 41a

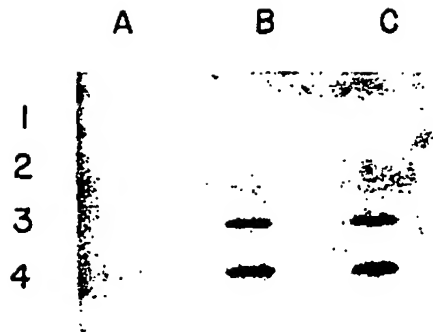


FIG. 41b

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**FIG. 41-**

Homology between the HCV polypeptide encoded by combined ORF of clones 141 through 39c) and the non-structural protein of the Dengue flavivirus (MNWVD1).

	10	20	30	40	50
HCV	EYVLLFLLADARVCSCLMMLLISQAEAALENLVIINAASLAGTHGLVSFLVFFCFA				
MNWVD1	AVSFVTLITGNMSFRDLGRVMVMVGATMTDDIGMGVTYLALLAFAKVRPTFAAGLLLRKL				
	130	140	150	160	170
	180				
	60	70	80	90	100
HCV	WYLGKQWPGAVYTFYGMWPLLLILLALPQRAYALDEVAASCGGVVLVGLMALTSPYY				
MNWVD1	TSKELMMTTIGIVLLSQSTIPETILELTDALALGMVLKMKVRKMEKYQLAVTIMAILCVF				
	190	200	210	220	230
	240				
	120	130	140	150	160
HCV	KRYISWCLWLOQYFLTRVEAQLHVWIPPLNVRGGRDAVILLHCAVHPTLVFDITKLLAV				
MNWVD1	NAVILQNAWKVSCTILAVSVSPLFLTSSQKADWIPLALTIKGLNPTAIF-LTTLRSTN				
	250	260	270	280	290
	180	190	200	210	220
HCV	FGPLWILQASLLKVPYF-VRVQGLLR-CAIARMMIGGHYVQVVIKLGALTGTYYVNH				
MNWVD1	KKRSWPLNEAIMAVGMVSILASSLLKNDIPMTGPLVAGGLTVCYV-LTGRSADLELERA				
	300	310	320	330	340
	350				
	240	250	260	270	280
HCV	TPLRDWAHNGLRDLAVAVEPVVFSQMETKLITWGADTAACGDIINGLPVSARRGREILG				
MNWVD1	ADVX-WEDQAEISGSSPILSITISE-DGSMSEKNEEEQTLTILIRTLGLVISG---LFP				
	360	370	380	390	400
	410				
	300	310	320	330	340
HCV	PADGMVSKGWRLLAPITAYAQTRGLLCIITSLTGRDKNQVEGEVQIVSTAAQTFATC				
MNWVD1	VSIPITAAAWYLWEVKQKQAGVLWDVSPFPVKGAELEDGAYRIKOKGILGYSQIGAGVY				
	420	430	440	450	460
	470				
	360	370	380	390	400
HCV	INGVCWTVYHGAGTRTIASPKGPVIOQMYTNVDQDLV---GWPAPQGSRSSTPCTCGSSD				
MNWVD1	KEGTFHTMWHVTRGAVIMHKGRIEPSWADVKKDLVSCGGGWKLEGEWKEGEEVQVLALE				
	480	490	500	510	520
	530				
	420	430	440	450	460
HCV	LYLVTRHADVIFVRRRGDSRGSLLSPRPISYLGSSGGPLLCAGHAVGIFRAAVCTRGV				
MNWVD1	PGKNPRAVQTKPGLFKTN--AGTIGAVSLDFSPGTSGSPIIDKKGVVGLYNGVVTTRSG				
	540	550	560	570	580
	590				
	480	490	500	510	520
HCV	AKAVDFIPVENLETMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSQKS--TKVPAAYAAQ				
MNWVD1	AYVSAIAQTEK--SIEDNPEIEDDIFRK--RKLTHDLHPGAGKTKRYLPAIVRGAIKR				
	600	610	620	630	640
	540	550	560	570	580

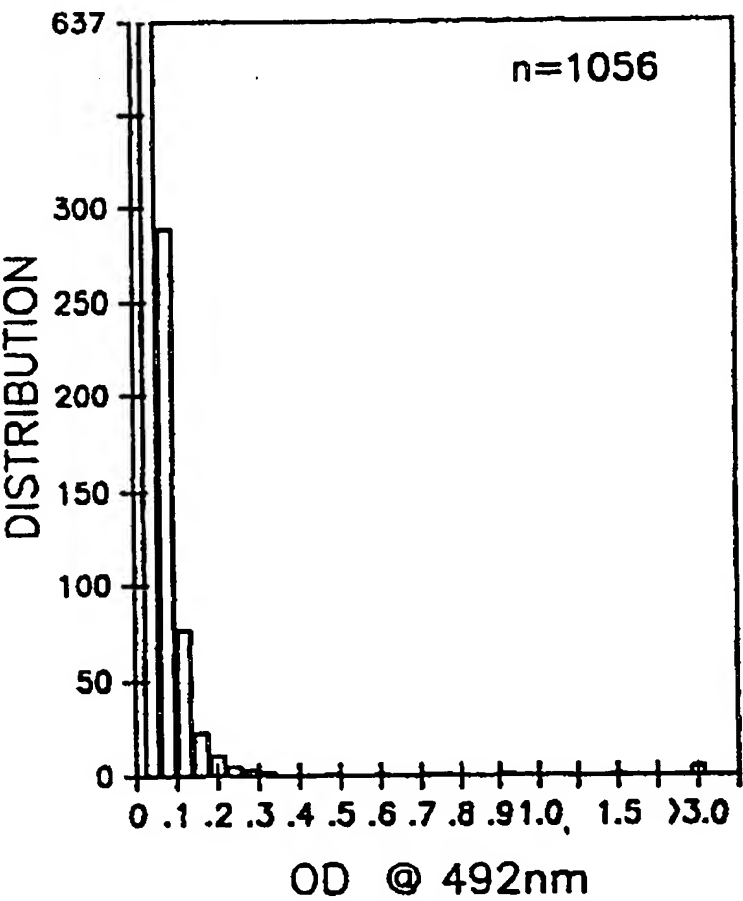
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HCV	GYKVLVLPNS—VAATLGFAYMSKAHGIDPNIRTGVRTITTGSPITYSTYKFLADGGC
MNWVD1	GLRTLILAPTRVVAAEMEEALRGLPIRYOTPAIRAHTGREIVDLNCHATTMRL—SPV
	650          660          670          680          690          700
HCV	590          600          610          620          630          640
	SGGAYDIIICDECHSTDATSI LGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEV
MNWVD1	RVPNYNLIIMDEAHFTDPASIAARGYISTRVE—MGEAAGIFMTATPPGSRD—PFPQSNAP
	710          720          730          740          750          760
HCV	650          660          670          680          690          700
	ALSTTGEIPFYGKAIPLEVIKGGRLIFCHSKKKCELAAKLVALGINAVAYYRGLDVSV
MNWVD1	IMDEEREIPERSWSSGHEWVTD FKGTIVWFVPSIKAGNDTAA CLRKNGKKVTQLSRKTFD
	770          780          790          800          810          820
HCV	710          720          730          740          750          760
	IPTSGDVVVVATDAIMTGYTGDFDSVIDCNTCVTQTVD FSLDPTFTIETITLPODAVSRT
MNWVD1	SEYVKTRINDWNFVVTTDISEMGANFKAERVIDPRRCMKPVILTDGEERVILAGPMPVTH
	830          840          850          860          870          880
HCV	770          780          790          800          810          820
	QRRGRGTGRGKPGIYRFVAPGERPSCMFDS SVLCECYDAGCAWYELTPAETT VRLRAYMNT
MNWVD1	SS

FIG. 41-2

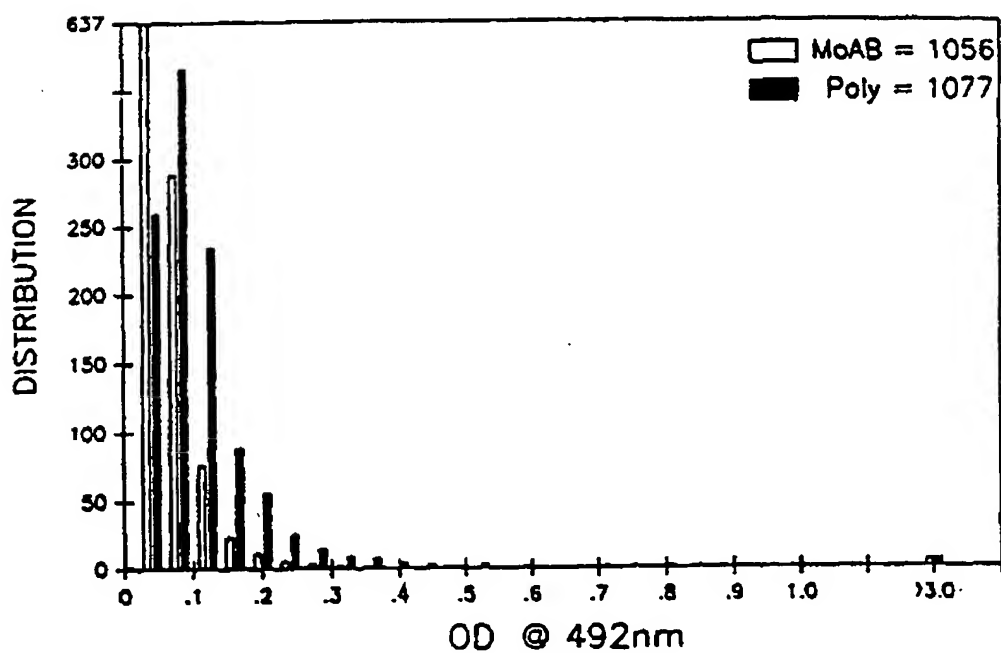
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**FIG. 43**  
**DISTRIBUTION OF RANDOM SAMPLES**  
**C100-3 Ag ELISA Preclinical Kit**  
**416ng C100/WELL, 2 HRS 37°C, 20ul SAMPLE**



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**FIG. 44**  
**Distribution of O.D. Values for**  
**Random Blood Donor Samples Tested with Two ELISA**  
**Configurations**  
C100-3 Ag ELISA MoAB vs Polyclonal



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**FIG. 45**

<u>Name</u>	<u>Common Sequence</u>	<u>Variable Sequence</u>
5'-3-1	AAGCTTGATCGAATTC	CGATCTTGC
-2		CGATCCTGC
-3		CGATCATGC
-4		CGATCGTGC
-5		CGAAGTTGC
-6		CGAAGCTGC
-7		AGATCTTGC
-8		AGATCCTGC
-9		AGATCATGC
-10		AGATCGTGC
-11		AGAAGTTGC
-12		AGAAGCTGC
-13		CGATCTTGT
-14		CGATCCTGT
-15		CGATCATGT
-16		CGATCGTGT
-17		CGAAGTTGT
-18		CGAAGCTGT
-19		AGATCTTGT
-20		AGATCCTGT
-21		AGATCATGT
-22		AGATCGTGT
-23		AGAAGTTGT
-24		AGAAGCTGT
-25		CGCTCTTGC
-26		CGCTCCTGC
-27		CGCTCATGC
-28		CGCTCGTGC
-29		CGCAGTTGC
-30		CGCAGCTGC
-31		CGCTCTTGT
-32		CGCTCCTGT
-33		CGCTCATGT
-34		CGCTCGTGT
-35		CGCAGTTGT
-36		CGCAGCTGT

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**FIG. 46-1** Translation of DNA k9-1

GlyCysProGluArgLeuAlaSerCysArgProLeuThrAspPheAspGlnGlyTrpGly  
 1 CAGGCTGTCCTGAGAGGCTAGCCAGCTGCCGACCCCTTACCGATTTTGACCAGGGCTGGG  
 GTCCGACAGGACTCTCCGATCGGTGACGGCTGGGAATGGCTAAACTGGTCCCGACCC

ProIleSerTyrAlaAsnGlySerGlyProAspGlnArgProTyrCysTrpHisTyrPro  
 61 GCCCTATCAGTTATGCCAACGGAAGCGGCCCGACCGCGCCCTACTGCTGGCACTACC  
 CGGGATAGTCAATACGGTTGCCTTCGCCGGGCTGGTCGCGGGGATGACGACCGTGATGG

ProLysProCysGlyIleValProAlaLysSerValCysGlyProValTyrCysPheThr  
 121 CCCCCAACCTTGCGGTATGTGCCCGCGAAGAGTGTGTGGTCCGGTATATTGCTTCA  
 GGGGTTTTGGAACGCCATAACACGGGCGCTTCTCACACACACCAGGCCATATAACGAAGT

ProSerProValValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGly  
 181 CTCCAGCCCCGTGGTGGTGGGAACGACCGACAGGTCCGGCGCGCCACCTACAGCTGGG  
 GAGGGTGGGGCACCACCACCCCTTGCTGGCTGTCCAGCCCGCGCGGGTGGATGTCCGACC

GluAsnAspThrAspValPheValLeuAsnAsnThrArgProProLeuGlyAsnTrpPhe  
 241 GTGAAATGATACGGACGCTCTTCGCTTAAACAATACAGGCCACCGCTGGCAATTGGT  
 CACCTTTTACTATGCCTGCAGAAGCAGGAATTGTATGGTCCGGTGGCGACCCGTTAACCA

GlyCysThrTrpMetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysVal  
 301 TCGGTTGTACCTGGATGAACCACTGGAATTCACCAAGTGTGCGGAGCGCCTCCTTGTG  
 AGCCAACTAGGACTACTTGAGTTGACCTAAGTGGTTTCACACGCTCGCGGAGGAACAC

IleGlyGlyAlaGlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisPro  
 361 TCATCGGAGGGCGGGCAACCAACCCCTGCACCTGCCCACTGATGCTTCGCAAGCATC  
 AGTAGCCTCCCCCGCGTGTGTGGGACGTGACGGGTGACTAACGAAGCGCTTCGTAG

AspAlaThrTyrSerArgCysGlySerGlyProTrpIleThrProArgCysLeuValAsp  
 421 CGGACGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGATCACACCCAGGTGCTGGTGG  
 GCCTGCGGTGTATGAGAGCCACGCCGAGGCCAGGGACCTAGTGTGGGTCCACGGACCAGC

-----  
 TyrProTyrArgLeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArg  
 481 ACTACCGTATAGGCTTTGGCATTATCCTTGTACCATCACTACCTATATTTAAATCA  
 TGATGGGCATATCCGAAACCGTAATAGGAACATGGTAGTTGATGTGATATAAATTTTAGT

-----  
 MetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGlu  
 541 GGATGTACGTGGGAGGGGTGAGCACAGGCTGGAAGCTGCCTGCACTGGACGCGGGGCG  
 CCTACATGCACCTCCCCAGCTCGTGTCCGACCTTCGACGGACGTTGACCTGCGCCCCGC

-----  
 ArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThr  
 601 AACGTTGCGATCTGGAAGATAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCACTA  
 TTGCAACGCTAGACCTTCTATCCCTGTCCAGGCTCGAGTCCGGCAATGACGACTGGTGAT

-----  
 GlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeuSerThrGlyLeuIle  
 661 CACAGTGGCAGGTCCTCCCGTGTTCCTTCACAACCTGCGAGCCTGTCCACCGGCTCA  
 GTGTACCGTCCAGGAGGGCACAAGGAAGTGTGGGACGCTCGGAACAGGTGGCCGGAGT

-----Overlap with Combined ORF of DNAs 12f through 15e-----  
 HisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyValGlySerSerIleAla  
 721 TCCACCTCCACCAGAACATTGTGGACGTGCAGTACTTGTACGGGGTGGGGTCAAGCATCG  
 AGGTGGAGGTGGTCTTGTAAACCTGCACGTGATGAACATGCCCCACCCAGTTGCTAGC

-----  
 SerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeuAlaAspAlaArg  
 781 CGTCCTGGGCCATTAAAGTGGGAGTACGTGCTCCTCCTGTTCTTCTGCTTGCAGACGGC  
 GCAGGACCCGGTAATTCACCCTCATGCAGCAGGAGGACAAGGAAGACGAACGTCTGCGCG

-----

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841 ValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsn  
 GCGTCTGCTCCTGCTTGTGGATGATGCTACTCATATCCCAAGCGGAAGCGGCTTTGGAGA  
 CGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTCGCCTTCGCCGAAACCTCT

901 LeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuVal  
 ACCTCGTAATACTTAATGCAGCATCCCTGGCCGGGACGCACGGTCTTGATCCTTCCTCG  
 TGGAGCATTATGAATTACGTCGTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGC

961 PhePheCysPheAlaTrpTyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPhe  
 TGTTCCTCTGCTTTGTCATGGTATCTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCT  
 ACAAGAAGACGAAACGTACCATAGACTTCCATTACCCACGGGCCCTCGCCAGATGTGGA

1021 TyrGlyMetTrpProLeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeu  
 TCTACGGGATGTGGCCTCTCCTCCTGCTCCTGTTGGCGTTGCCCCAGCGGGCGTACGGC  
 AGATGCCCTACACCGGAGAGGAGGACGAGGACAACCGCAACGGGGTTCGCCGATGCGCG

1081 AspThrGluValAlaAlaSerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThr  
 TGCACACGGAGGTGGCCGCTCGTGTGGCGGTGTGTTCTCGTCCGGTGTATGGCGCTAA  
 ACCTGTGCCCTCCACCGGCGCAGCACACCGCCACAACAAGAGCAGCCCAACTACCGCGATT

1141 LeuSerProTyrTyrLysArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeu  
 CTCTGTCACCATATTACAAGCGCTATATCAGCTGGTGTGTTGGTGGCTTCAGTATTTTC  
 GAGACAGTGGTATAATGTTCCGATATAGTCGACCACGAACACCACCGAAGTCATAAAAG

1201 ThrArgValGluAlaGlnLeuHisValTrpIleProProLeuAsnValArgGlyGlyArg  
 TGACCAGAGTGAAGCGCAACTGCACGTGTGGATTCCCCCCTCAACGTCCGAGGGGGGC  
 ACTGGTCTCACCTTCGCGTTGACGTGCACACCTAAGGGGGGAGTTGCAGGCTCCCCCG

1261 AspAlaValIleLeuLeuMetCysAlaValHisProThrLeuValPheAspIleThrLys  
 GCGACGCTGTCTTACTCATGTGTGCTGTACACCGACTCTGGTATTTGACATCACCA  
 CGCTGCGACAGTAGAATGAGTACACGACATGTGGGCTGAGACCATAAACTGTAGTGGT

1321 LeuLeuLeuAlaValPheGlyProLeuTrpIleLeuGlnAla  
 AATTGCTGCTGGCCGCTCTCGGACCCCTTTGGATTCTTCAAGCCAG  
 TTAACGACGACCGGCAGAACCTGGGGAAACCTAAGAAGTTCGGTC

FIG. 46-2

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FIG. 47-1 COMBINED ORF OF DNAs K9-1 through 15

GlyCysProGluArgLeuAlaSerCysArgProLeuThrAspPheAspGlnGlyTrpGly  
 1 CAGGCTGTCTCTAGAGGCTAGCCAGCTGCCGACCCCTACCGATTTTGACCAGGGCTGGG  
 GTCCGACAGGACTCTCCGATCGGTGACGGCTGGGAATGGCTAAAACTGGTCCCGACCC  
 ProIleSerTyrAlaAsnGlySerGlyProAspGlnArgProTyrCysTrpHisTyrPro  
 61 GCCCTATCAGTTATGCCAAGCGGCGCCGACGAGCGCCCTACTGCTGGCACTACC  
 CGGGATAGTCAATACGGTTGCCTTCGCCGGGGCTGGTCGGGGGATGACGACCGTGATGG  
 ProLysProCysGlyIleValProAlaLysSerValCysGlyProValTyrCysPheThr  
 121 CCCCAAAACCTTGGCGTATTGTGCCGCGAAGAGTGTGTGGTCCGGTATATTGCTTCA  
 GGGGTTTGGAAAGCCATAACACGGGCGCTTCTCACACACACCAGGCCATATAACGAAGT  
 ProSerProValValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGly  
 181 CTCCCAGCCCCGTGGTGGTGGGAACGACGACAGGTGGGGGCGCCACCTACAGCTGGG  
 GAGGTGCGGGGCACCACCACCTTGCTGGCTGTCCAGCCCGCGGGGTGGATGTCGACCC  
 GluAsnAspThrAspValPheValLeuAsnAsnThrArgProProLeuGlyAsnTrpPhe  
 241 GTGAAAATGATACGGACGTCTTCGTCTTAAACAATACCAGGCCACCGCTGGGCAATTGGT  
 CACTTTTACTATGCCTGCAGAAGCAGGAATTGTTATGGTTCGGTGGCGACCCGTTAACCA  
 GlyCysThrTrpMetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysVal  
 301 TCGGTTGTACCTGGATGAACCTCACTGGATTCAACAAAGTGTGCGGAGCGCTCCTGTG  
 AGCCAAACATGACCTACTTGAGTTGACCTAAGTGGTTTCACAGCCCTCGCGGAGGAACAC  
 IleGlyGlyAlaGlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisPro  
 361 TCATCGGAGGGGCGGGCAACAACACCCCTGCACTGCCCTACTGATGCTTCCGCAAGCATC  
 AGTAGCCTCCCGCCCGTTGTTGTGGGACGTGACGGGTGACTAACGAAGCGTTCTGTAG  
 AspAlaThrTyrSerArgCysGlySerGlyProTrpIleThrProArgCysLeuValAsp  
 421 CGGACGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGATCACACCAGGTGCCTGGTCG  
 GCCTGCGGTGTATGAGAGCCACGCCGAGGCCAGGGACCTAGTGTGGGTCCACGGACCAGC  
 TyrProTyrArgLeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArg  
 481 ACTACCCGTATAGGCTTTGGCATTATCCTTGTACCATCAACTACACCATATTAAATCA  
 TGATGGGCATATCCGAACCGTAATAGGAACATGTTAGTTGATGTGTTATAAATTTTAGT  
 MetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGlu  
 541 GGATGTACGTGGGAGGGGTGGAACACAGGCTGGAAGCTGCCTGCAACTGGACGCGGGCGC  
 CCTACATGCACCTCCCGAGCTTGTGTCCGACCTTCGACGGACGTTGACCTGCGCCCGC  
 ArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThr  
 601 AACGTTGCGATCTGGAAGACAGGGACAGGTCCGAGCTCAGCCCGTTACTGTGACCACTA  
 TTGCAACGCTAGACCTTCTGTCCCTGTCCAGGCTCGAGTCGGGCAATGACGACTGGTGAT  
 GlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeuSerThrGlyLeuIle  
 661 CACAGTGGCAGGTCTCCCGTGTTCCTTCACAACCTACCAGCCTGTCCACCGGGCTCA  
 GTGTACCGTCCAGGAGGGCACAAGGAAGTGTGGGATGGTCGGAACAGGTGGCCGGAGT  
 HisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyValGlySerSerIleAla  
 721 TCCACCTCCACCAGAACATTGTGGACGTGCAGTACTTGTACGGGGTGGGGTCAAGCATCG  
 AGGTGGAGGTGGTCTTGTAAACCTGCACGTGATGAACATGCCCCACCCAGTTCTGTAGC  
 SerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeuAlaAspAlaArg  
 781 CGTCTGGGCCATTAAAGTGGAGTACGTCTGTTCTCTGTTCTCTGCTTGACAGCGCGC  
 GCAGGACCGGTAATTACCCCTCATGCAGCAAGAGGACAAGGAAGCAACGCTCTGCGCG  
 ValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsn  
 841 GCGTCTGCTCCTGCTTGTGGATGATGCTACTCATATCCCAAGCGGAGGCGGCTTTGGAGA  
 CGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTGCGCTCCGCCGAACCTCT  
 LeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuVal  
 901 ACCTCGTAATACTTAATGCAGCATCCCTGGCCGGGACGACGCTTGTATCCTTCTCG  
 TGGAGCATTATGAATTACGTCTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGC

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PhePheCysPheAlaTrpTyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPhe  
 961 TGTTCCTCTGCTTTGCATGGTATTTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCT  
 ACAAGAAGACGAAACGTACCATAAACTTCCCATTCACCCAAGGGCTCGCCAGATGTGGA  
  
 TyrGlyMetTrpProLeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeu  
 1021 TCTACGGGATGTGGCCTCTCCTCCTGCTCCTGTGGCGTTGCCCGAGCGGGCGTACGGCG  
 AGATGCCCTACACCGAGAGGAGGACGAGGACAACCGCAACGGGGTGGCCCGCATGGCGG  
  
 AspThrGluValAlaAlaSerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThr  
 1081 TGGACACGGAGGTGGCGCGTGTGTGGCGGTGTTGTTCTCGTGGGTGTATGGCGCTGA  
 ACCTGTGCCTCCACCGCGCAGCACACCGCCACACACAGAGCAGCCCACTACCGGACT  
  
 LeuSerProTyrTyrLysArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeu  
 1141 CTCTGTCAACCATATTACAAGCGCTATATCAGCTGGTGTGTGTGGTGGCTTCAGTATTTTC  
 GAGACAGTGGTATAATGTTCCGATATAGTCGACCACGAACACCGAAGTCATAAAAG  
  
 ThrArgValGluAlaGlnLeuHisValTrpIleProProLeuAsnValArgGlyGlyArg  
 1201 TGACAGAGTGAAGCGCACTGCACGTGTGGATTCCCCCTCAACGTCGAGGGGGGCG  
 ACTGTCTCTACCTTCGCGTTGACGTGCACACCTAAGGGGGGGAGTTGCAGGCTCCCCCG  
  
 AspAlaValIleLeuLeuMetCysAlaValHisProThrLeuValPheAspIleThrLys  
 1261 GCGACGCCGTCTACTCTATGTGTGTGTACACCGACTCTGGTATTGACATCAACCA  
 CGCTGCGGCAGTAGAATGAGTACACACGACATGTGGGTGAGACCATAAACTGTAGTGGT  
  
 LeuLeuLeuAlaValPheGlyProLeuTrpIleLeuGlnAlaSerLeuLeuLysValPro  
 1321 AATTGCTGCTGGCGCTCTTCGGACCCCTTTGGATTCTTCAAGCCAGTTTGCTTAAAGTAC  
 TTAACGACGACCGGAGAGCCTGGGGAAACCTAAGAAGTTTCGGTCAACGAATTTTCATG  
  
 TyrPheValArgValGlnGlyLeuLeuArgPheCysAlaLeuAlaArgLysMetIleGly  
 1381 CCTACTTTGTGCGCGTCCAAGGCCTTCTCCGGTCTGCGCGTTAGCGCGGAAGATGATCG  
 GGATGAAACACGCGCAGGTTCCGGAAGAGGCCAAGACGCGCAATCGCGCTTCTACTAGC  
  
 GlyHisTyrValGlnMetValIleIleLysLeuGlyAlaLeuThrGlyThrTyrValTyr  
 1441 GAGGCCATTACGTGCAATGGTCATCATTAAAGTAGGGGCGCTTACTGGCACCTATGTTT  
 CTCCGTAATGCACGTTTACAGTAGTAATTCATCCCGGAATGACCGTGGATACAAA  
  
 AsnHisLeuThrProLeuArgAspTrpAlaHisAsnGlyLeuArgAspLeuAlaValAla  
 1501 ATAACCATCTCACTCCTCTTCGGAGCTGGGCGCACACGGCTTGGAGATCTGGCGGTGG  
 TATTGGTAGAGTGAGGAGAAGCCTGACCCGCGTGTGCCGAACGCTCTAGACCGGCACC  
  
 ValGluProValValPheSerGlnMetGluThrLysLeuIleThrTrpGlyAlaAspThr  
 1561 CTGTAGAGCCAGTCTGCTCTCTCCAAATGGAGACCAAGCTCATCACGTGGGGGGCAGATA  
 GACATCTCGGTCAGCAGAAGAGGGTTTACCTCTGGTTCGAGTAGTGACCCCCCGTCTAT  
  
 AlaAlaCysGlyAspIleIleAsnGlyLeuProValSerAlaArgArgGlyArgGluIle  
 1621 CCGCCGCGTGGCGTGACATCATCAACGGCTTGCTGTCTCCGCCCGCAGGGGCGGGGAGA  
 GGCGGCGCACGCCACTGTAGTAGTTGCCGAACGACAAAGGCGGGCGTCCCCGGCCCTCT  
  
 LeuLeuGlyProAlaAspGlyMetValSerLysGlyTrpArgLeuLeuAlaProIleThr  
 1681 TACTGCTCGGGCCAGCGATGGAATGGTCTCCAGGGGTGGAGGTGTCTGGCGCCCATCA  
 ATGACGAGCCCGTCCGCTACCTTACCAGAGGTTCACCACTCCAACGACCGGGGTAGT  
  
 AlaTyrAlaGlnGlnThrArgGlyLeuLeuGlyCysIleIleThrSerLeuThrGlyArg  
 1741 CGGCGTACGCCCGCAGACAAAGGGCTCTAGGGTGCATAATCACCAGCCTAACTGGCC  
 GCGCATGCGGTCTGTCTTCCCGGAGGATCCACGTATTAGTGGTGGATTGACCGG  
  
 AspLysAsnGlnValGluGlyGluValGlnIleValSerThrAlaAlaGlnThrPheLeu  
 1801 GGGACAAAACCAAGTGGAGGGTGAGGTCCAGATTGTGTCAACTGCTGCCAAACCTTCC  
 CCTGTCTTTTGGTTACCTCCCACTCCAGCTCTAACACAGTTGACGACGGGTTTGAAGG  
  
 AlaThrCysIleAsnGlyValCysTrpThrValTyrHisGlyAlaGlyThrArgThrIle  
 1861 TGGCAACGTGCATCAATGGGGTGTCTGACTGTCTACACGGGGCCGGAAACGAGGACCA  
 ACCGTTGCACGTAGTTACCCACACGACCTGACAGATGGTGCCCGGGCTGTCTCTGGT  
  
 AlaSerProLysGlyProValIleGlnMetTyrThrAsnValAspGlnAspLeuValGly

FIG. 47-2

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1921 TCCCGTCACCCAAGGGTCTCTGTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGG  
AGCGCAGTGGGTTCCAGGACAGTAGGTCTACATATGGTTACATCTGGTTCTGGAACACC

TrpProAlaProGlnGlySerArgSerLeuThrProCysThrCysGlySerSerAspLeu  
1981 GCTGGCCCGCTCCGCAAGGTAGCCGCTCATTGACACCTGCACTTGGCGGTCCTCGGACC  
CGACCGGGCGAGGCGTTCCATCGGCGAGTAACGTGGGACGTGAACGCCGAGGAGCCTGG

TyrLeuValThrArgHisAlaAspValIleProValArgArgArgGlyAspSerArgGly  
2041 TTTACCTGGTCACGAGGCACGCGATGTCAATCCCGTGGCGGGGGGTGATAGCAGGG  
AAATGGACCACTGCTCCGTGCGGTACAGTAAGGGCACGCGGCGCCCACTATCGTCCC

SerLeuLeuSerProArgProIleSerTyrLeuLysGlySerSerGlyGlyProLeuLeu  
2101 GCAGCCTGCTGTGCGCCGCGCCATTTCTACTTGAAGGCTCCTCGGGGGTCCGCTGT  
CGTCGGACGACAGCGGGCGGGTAAGGATGAACCTTCGAGGAGCCCCAGGCGACA

CysProAlaGlyHisAlaValGlyIlePheArgAlaAlaValCysThrArgGlyValAla  
2161 TGTGCCCGCGGGGCACGCGGTGGGCATATTTAGGGCCGCGGTGTGCACCCGTGGAGTGG  
ACACGGGGCGCCCGTGGCGCACCCGTATAAATCCGCGGCCACACGTGGGCACCTCACC

LysAlaValAspPheIleProValGluAsnLeuGluThrThrMetArgSerProValPhe  
2221 CTAAGGCGGTGGACTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCGGTGT  
GATTCCGCCACCTGAAATAGGGACACCTCTTGATCTCTGTGGTACTCCAGGGGCCACA

ThrAspAsnSerSerProProValValProGlnSerPheGlnValAlaHisLeuHisAla  
2281 TCACGGATAACTCCTCTCCACCACTAGTGGCCAGAGCTTCCAGGTGGCTCACCTCCATG  
AGTGCTATTGAGGAGAGGTGGTCATCACGGGGTCTCGAAGGTCCACCGAGTGGAGGTAC

ProThrGlySerGlyLysSerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLys  
2341 CTCGCCACAGGCAGCGGCAAAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATA  
GTGGGTGCTGCGCGTTTCGTGGTTCCAGGGCGGACGTATACGTGAGTCCCGAT

ValLeuValLeuAsnProSerValAlaAlaThrLeuGlyPheGlyAlaTyrMetSerLys  
2401 AGGTGCTAGTACTCAACCCCTCTGTGTGCTGCAACACTGGGCTTTGGTGTCTACATGTCCA  
TCCAGATCATGAGTTGGGAGACAACGACGTGTGACCCGAAACCAAGATGTACAGGT

AlaHisGlyIleAspProAsnIleArgThrGlyValArgThrIleThrThrGlySerPro  
2461 AGGCTCATGGGATCGATCCTAACATCAGGACCGGGGTGAGAACCAATTACCACTGGCAGCC  
TCGAGTACCCTAGCTAGGATTGTAGTCTCGCCCACTCTGTGTTAATGCTGACCGTCCG

IleThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyr  
2521 CCATCAGTACTCCACTACGGCAAGTTCCTTGGCGACGGCGGGTCTCGGGGGCGGCT  
GGTAGTGCATGAGGTGGATGCCGTTCAAGGAACGGCTGCGCCCAAGAGCCCCCGGAA

AspIleIleIleCysAspGluCysHisSerThrAspAlaThrSerIleLeuGlyIleGly  
2581 ATGACATAATAATTTGTGACGAGTGCCACTCCACGGATGCCACATCCATCTTGGGCATCG  
TACTGTATTATTAAACACTGCTCACGCTGAGGTGCCACGCTGTAGGTAGAACCCTAGC

ThrValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThr  
2641 GCACTGTCCTTGACCAAGCAGAGACTCGGGGGCGAGACTGGTTGTGCTCGCCACCGCCA  
CGTGACAGGAACGTGGTCTCTGACGCCCCCGCTCTGACCAACAGAGCGGTGGCGGT

ProProGlySerValThrValProHisProAsnIleGluGluValAlaLeuSerThrThr  
2701 CCCCTCCGGGCTCCGTCAGTGTGCCCCATCCCAACATCGAGGAGGTGCTCTGTCCACCA  
GGGGAGGCCCGAGGCAGTGACAGGGGTAGGGTTGTAGCTCTCCAACGAGACAGGTGGT

GlyGluIleProPheTyrGlyLysAlaIleProLeuGluValIleLysGlyGlyArgHis  
2761 CCGGAGAGATCCCTTTTACGGCAAGGCTATCCCCCTCGAAGTAATCAAGGGGGGAGAC  
GGCTCTCTAGGGAAAAATGCCGTTCCGATAGGGGGAGCTCATTAGTCCCCCTCTG

LeuIlePheCysHisSerLysLysLysCysAspGluLeuAlaAlaLysLeuValAlaLeu  
2821 ATCTCATCTTCTGTCAATCAAAGAAGTGCGACGAACCTGCCGCAAGCTGGTCGCAT  
TAGAGTAGAAGACAGTAAGTTTCTTTCACGCTGCTTGACGGCGTTTCGACCAGCGTA

GlyIleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerValIleProThrSerGly  
2881 TGGGCATCAATGCGTGGCTACTACCGGCTTGTGACGTGTCCGTCATCCCGACCGAGCG  
ACCCGTAGTTACGGCACCGGATGATGGCGCCAGAACTGCACAGGCAGTAGGGCTGTGCG

FIG. 47-3

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2941 AspValValValValAlaThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSer  
 GCGATGTTGTCGTCGTGGCAACCGATGCCCTCATGACCGGCTATACCGGCGACTTCGACT  
 CGCTACAACAGCAGCACCGTTGGCTACGGGAGTACTGGCCGATATGGCCGTGAAGCTGA  
 3001 ValIleAspCysAsnThrCysValThrGlnThrValAspPheSerLeuAspProThrPhe  
 CGGTGATAGACTGCAATACGTGTGTACCCAGACAGTCGATTTCAGCCTTGACCCTACCT  
 GCCACTATCTGACGTTATGCACACAGTGGGTCGTGTCAGCTAAAGTCGGAAGTGGGATGGA  
 3061 ThrIleGluThrIleThrLeuProGlnAspAlaValSerArgThrGlnArgArgGlyArg  
 TCACCATTGAGACAATCAAGCTCCCCAGGATGCTGTCTCCCGCACTCAAGTCGGGGCA  
 AGTGGTAACCTCTGTTAGTGGAGGGGGTCTACGACAGAGGGCGTGAGTTGCAGCCCCGT  
 3121 ThrGlyArgGlyLysProGlyIleTyrArgPheValAlaProGlyGluArgProSerGly  
 GGACTGGCAGGGGAAGCCAGGCATCTACAGATTGTGGCACCGGGGAGCGCCCCCTCG  
 CCTGACCGTCCCTTCGGTCCGTAGATGTCTAAACACCGTGGCCCCCTCGGGGGAGGC  
 3181 MetPheAspSerSerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeu  
 GCATGTCGACTGTCGCTCTCTGTGAGTGCTATGACGAGGCTGTGCTTGGTATGAGC  
 CGTACAAGCTGAGCAGGCAGGAGACACTCACGATACTGCGTCCGACACGAACCATACTCG  
 3241 ThrProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThrProGlyLeuProVal  
 TCACGCCCGCGAGACTACAGTTAGGCTACGAGCGTACATGAACACCCCGGGCTTCGCG  
 AGTGGGGGGGCTCTGATGTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGGC  
 3301 CysGlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeuThrHisIleAspAla  
 TGTGCCAGGACCATCTTGAATTTGGGAGGGCGTCTTTACAGGCTCACTCATATAGATG  
 ACACGGTCTCTGGTAGAATTAACCCCTCCCGCAGAAATGTCGGAGTGAGTATATCTAC  
 3361 HisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGln  
 CCCACTTTCTATCCAGACAAAGCAGAGTGGGGAGAACCCTTCCTTACCTGGTAGCGTACC  
 GGGTGAAGATAGGGTCTGTTTCGTCTACCCCTCTTGAAGGAATGGACCATGCGATGG  
 3421 AlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCys  
 AAGCCACCGTGTGCGTAGGGCTCAAGCCCTCCCCATCGTGGGACAGATGTGGAAGT  
 TTCGGTGGCACACCGATCCCGAGTTCGGGGAGGGGTAGCACCTGGTCTACACCTTCA  
 3481 LeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAla  
 GTTGTATTGCGCTCAAGCCACCTCCATGGGCCAACCCCTGCTATACAGACTGGGCG  
 CAACTAAGCGGAGTTCGGGTGGGAGGTACCGGTTGTGGGGACGATATGTCGACCGC  
 3541 ValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIleMetThrCysMetSer  
 CTGTTTCAGAAATGAATCACCTGACGCAACCCAGTCAACAAATACATCATGACATGCATGT  
 GACAAGTCTTACTTTAGTGGGACTGCGTGGGTGAGTGGTTTATGTAGTACTGTACGTACA  
 3601 AlaAspLeuGluValValThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeu  
 CGGCCGACCTGGAGGTGTCACGAGCACCTGGGTGCTGTTGGCGGCTCCTGGCTGCTT  
 GCGGCTGGACCTCCAGCAGTGTGCTGAGCCACGAGCAACGCGCAGGACCGACGAA  
 3661 AlaAlaTyrCysLeuSerThrGlyCysValIleValGlyArgValValLeuSerGly  
 TGGCCGCGTATTGCTGTCAACAGGCTGCGTGGTCATAGTGGCAGGTCGCTTGTCCG  
 ACCGGCGCATAACGGACAGTTGTCCGACGACAGTATCACCGTCCAGCAGAACAGGC  
 3721 LysProAlaIleIleProAspArgGluValLeuTyrArgGluPheAspGluMetGluGlu  
 CGAAGCCCGCAATCATACCTGACAGGCAAGTCTCTACCGAGAGTTGATGAGATGGAAG  
 CCTTCGGCCGTTAGTATGGACTGTCCCTTACAGGAGATGGCTCTCAAGCTACTCTACCTTC  
 3781 CysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPheLysGln  
 AGTGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGCTCGCCGAGCAGTTCAAGC  
 TCACGAGAGTGTGAATGGCATGTAGCTGTTCCCTACTACGAGCGGCTCGTCAAGTTCG  
 3841 LysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaProAlaVal  
 AGAAGGCCCTCGGCTCTGAGACCGCTCCGTCAGGCAAGGTTATCGCCCTGCTG  
 TCTTCGGGAGCGGAGGACGCTGGGCGAGGCGAGTCCGCTCCAAATAGCGGGGAGCAGC  
 GlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPheIleSer

FIG. 47-4

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3901 TCCAGACCAACTGGCAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAACCTTCATCA  
AGGTCTGGTIGACCGTTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAGTAGT

GlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAlaSerLeu  
3961 GTGGGATACAATACTTGGCGGGCTTGTCACCGCTGCCTGGTAACCCCGCATTTGCTTCAT  
CACCTATGTTATGAACCGCCGACAGTTGCGACGGACCATTTGGGCGGTAACGAAGTA

MetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsn  
4021 TGATGGCTTTTACAGCTGCTGTACCAGCCACTAACCACTAGCCAAACCTCCTCTTCA  
ACTACCGAAATGTGACGACAGTGGTGGGTGATTGGTGTATCGGTTTGGGAGGAGAAGT

IleLeuGlyGlyTrpValAlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheVal  
4081 ACATATTGGGGGGTGGGTGGCTGCCAGCTGCGCGCCCGGGTGGCGCTACTGCTTTG  
TGTATAACCCCCACCCACCGACGGTTCGAGCGCGGGGGCCACGGCGATGACGGAAC

GlyAlaGlyLeuAlaGlyAlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAsp  
4141 TGGGCGCTGGCTTAGCTGGCGCCGOCATGCGCAGTGTGGACTGGGAAGGTCCCTCATAG  
ACCGCGAOCGAATCGACCGCGGGTAGCCGTCAACCTGACCCCTTCCAGGAGTATC

IleLeuAlaGlyTyrGlyAlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSer  
4201 ACATCCTTGCAGGGTATGGCGCGGGCGTGGCGGAGCTCTGTGGCATTCAAGATCATGA  
TGTAGGAACGTCCCATACCGCGCCCGCACCCTCGAGAACACCGTAAGTTCTAGTACT

GlyGluValProSerThrGluAspLeuValAsnLeuLeuProAlaIleLeuSerProGly  
4261 GCGGTGAGGTCCCTCCACCGAGGACCTGGTCAATCTACTGCCCGCCATCTCTCGCCCG  
CGCCACTCCAGGGGAGGTGCTCTGGACAGTTAGATGACGGGGGTAGGAGAGCGGGC

AlaLeuValValGlyValValCysAlaAlaIleLeuArgArgHisValGlyProGlyGlu  
4321 GAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCAATACTGCCCGGCCACGTGGCCCGGGCG  
CTCGGGAGCATCAGCCGACACAGACAGTCTGTTATGACGCGCGCTGCACCGGGGCCCGC

GlyAlaValGlnTrpMetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSer  
4381 AGGGGGCAGTCAGTGGATGAACCGCTGATAGCCTTCGCTCCCGGGGAACCATGTTT  
TCCCCGCTCAGTCACTACTTGGCCGACTATCGGAAGCGGAGGCCCCCTTGGTACAAA

ProThrHisTyrValProGluSerAspAlaAlaAlaArgValThrAlaIleLeuSerSer  
4441 CCCCCACGCACTACGTGCCGAGAGCGATGCAGCTGCCCCGCTACTGCCATACTCAGCA  
GGGGGTGCGTGATGCACGGCTCTCGCTACGTGACGGGCGCAGTGACGGTATGAGTCGT

LeuThrValThrGlnLeuLeuArgArgLeuHisGlnTrpIleSerSerGluCysThrThr  
4501 GCCTCACTGTAAACCCAGCTCCTGAGGCGACTGCACCACTGATAGCTCGGAGTGTACCA  
CGGAGTGACATTGGGTGCGAGGACTCGCTGACGTGGTCACTATTGAGCCTCACATGGT

ProCysSerGlySerTrpLeuArgAspIleTrpAspTrpIleCysGluValLeuSerAsp  
4561 CTCCATGCTCCGGTTCTTGGCTAAGGACATCTGGGACTGATATGCGAGGTGTTGAGCG  
GAGGTACGAGGCCAAGGACCGATTCCCTGTAGACCTGACCTATACGCTCCACAACCTCGC

PheLysThrTrpLeuLysAlaLysLeuMetProGlnLeuProGlyIleProPheValSer  
4621 ACTTTAAGACCTGGCTAAAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCTTTGTGT  
TGAAATTCTGGACCGATTTTGTATTGAGTACGGTGTGACGGACCTAGGGGAACACA

CysGlnArgGlyTyrLysGlyValTrpArgValAspGlyIleMetHisThrArgCysHis  
4681 CCTGCCAGCGCGGTATAAGGGGTCTGGCGAGTGGACGGCATCATGCACACTCGCTGCC  
GGACGGTGGCGCCATATTCCCCAGACCGCTACCTGCGGTAGTACGTGTGAGCGACGG

CysGlyAlaGluIleThrGlyHisValLysAsnGlyThrMetArgIleValGlyProArg  
4741 ACTGTGGAGCTGAGATCACTGGACATGTCAAAAACGGGACGATGAGGATCGTCGGTCCTA  
TGACACCTCGACTCTAGTGACCTGTACAGTTTTTGCCTGTACTCTTAGCAGCCAGGAT

ThrCysArgAsnMetTrpSerGlyThrPheProIleAsnAlaTyrThrThrGlyProCys  
4801 GGACCTGCAGGAACATGTGGAGTGGGACCTTCCCATTAATGCTTACACCACGGGGCCCT  
CCTGGACGTCTTGTACACCTACCCCTGGAAGGGTAATTACGGATGTGGTCCCGGGGA

ThrProLeuProAlaProAsnTyrThrPheAlaLeuTrpArgValSerAlaGluGluTyr  
4861 GTACCCCTTCTCTGCCCGAACTACAGTTGCGCTATGGAGGGTGTCTGCAGAGGAAT  
CATGGGGGAAGGAOGCGCTGATGTGCAAGCGGATACCTCCACAGACGTCTCCTTA

FIG. 47-5

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5881 GTGAGGCCAACGCGGAGGATGTCGTGTGCTCAATGTCCTACTCTGGACAGGCGCAC  
CACTCCGGTTGCGCCTCTACAGCACACGAGTTACAGAATGAGAACCTGTCCGCGTG

ValThrProCysAlaAlaGluGlnLysLeuProIleAsnAlaLeuSerAsnSerLeu

5941 TCGTACCCCGTGGCGCGGGAAGAACAGAACTGCCATCAATGCACTAAGCAACTCGT  
AGCAGTGGGGCAGCGGCGCCTTCTTGTCTTTGACGGGTAGTTACGTGATTCTGTGAGCA

LeuArgHisHisAsnLeuValTyrSerThrThrSerArgSerAlaCysGlnArgGlnLys

6001 TGCTACGTACCCACAATTGGTGTATTCCACCACCTCAGCAGTGTCTGCCAAAGGCAGA  
ACGATGCAGTGGTGTAAACCACATAAGGTGGTGGAGTGGTTCACGAACGGTTTCCGTCT

LysValThrPheAspArgLeuGlnValLeuAspSerHisTyrGlnAspValLeuLysGlu

6061 AGAAAGTCACATTGACAGACTGCAAGTTCTGGACAGCATTACCAGGACGTACTCAAGG  
TCTTTCAGTGTAAACTGTCTGACGTTCAAGACCTGTGGTAATGGTCTGTCATGAGTTCC

ValLysAlaAlaAlaSerLysValLysAlaAsnLeuLeuSerValGluGluAlaCysSer

6121 AGCTTAAGCAGCGGGCTCAAGTGAAGGCTAAGTGTATCGTACAGGAAGCTTGCA  
TCCAATTTGCTGCGCGCAGTTTTCACCTCCGATGAACGATAGGCATCTCCTTCGAACT

LeuThrProProHisSerAlaLysSerLysPheGlyTyrGlyAlaLysAspValArgCys

6181 GCCTGACGCCCCCACTCAGCCAAATCCAAGTTTGGTATGGGGCAAAGACGTCGGTT  
CGGACTGCGGGGTGTGAGTCCGTTTAGTTCAAACCAATACCCGTTTCTGACGGCAA

HisAlaArgLysAlaValThrHisIleAsnSerValTrpLysAspLeuLeuGluAspAsn

6241 GCCATGCCAGAAAGCGGTAAACCATCACTCCGTGTGGAAGACCTTCTGGAAGACA  
CGGTACGGTCTTTCGGCATTTGGGTGTAGTTGAGGCACACCTTCTGGAAGACCTTCTGT

ValThrProIleAspThrThrIleMetAlaLysAsnGluValPheCysValGlnProGlu

6301 ATGTAACACCAATAGACACTACCATCATGGCTAAGAACGAGGTTTCTGCGTTCAGCCTG  
TACATTGTGGTTATCTGTGATGGTAGTACGATTCTTGCTCCAAAGACGCAAGTCGGAC

LysGlyGlyArgLysProAlaArgLeuIleValPheProAspLeuGlyValArgValCys

6361 AGAAGGGGGGTCTGTAAGCCAGCTCGTCTCATCGTGTTCGCCATCTGGGCGTGGCGGTGT  
TCTTCCCCCAGCATTCGGTCCAGCAGAGTAGCACAAGGGCTAGACCCGCACGGCACA

GluLysMetAlaLeuTyrAspValValThrLysLeuProLeuAlaValMetGlySerSer

6421 GCGAAAAGATGGCTTTGTACGACGTGGTTACAAAGCTCCCTTGGCCGTGATGGGAAGCT  
CGCTTTTCTACCGAAACATGCTGCACCAATGTTTCGAGGGGAACCGGCACTACCTTCGA

TyrGlyPheGlnTyrSerProGlyGlnArgValGluPheLeuValGlnAlaTrpLysSer

6481 CCTACGGATTCCAATACTCACCAGGACAGCGGGTGAATTCCTCGTGCAAGCGTGGAGT  
GGATGCCCTAAGGTTATGAGTGGTCTGTGCGCCCACTTAAGGAGCACGTTCCGACCTTCA

LysLysThrProMetGlyPheSerTyrAspThrArgCysPheAspSerThrValThrGlu

6541 CCAAGAAACCCCAATGGGGTTCTCGTATGATACCGCTGCTTTGACTCCACAGTCACTG  
GGTCTTTTGGGGTTACCCCAAGAGCATACTATGGGCGACGAACTGAGGTGTCAAGTAC

SerAspIleArgThrGluGluAlaIleTyrGlnCysCysAspLeuAspProGlnAlaArg

6601 AGAGCGACATCCGTACGGAGGAGGCAATCTACCAATGTTGTGACCTCGACCCCAAGCCC  
TCTCGCTGTAGGCATGCCTCTCCGTTAGATGGTTACAACACTGGAGCTGGGGTTCCGG

ValAlaIleLysSerLeuThrGluArgLeuTyrValGlyGlyProLeuThrAsnSerArg

6661 GCGTGGCCATCAAGTCCCTCAGCAGAGGCTTATGTTGGGGGCCCTCTTACCAATTCAA  
CGCACCGGTAGTTACGGAGTGGCTCTCCGAATACAACCCCGGAGAAATGGTTAAGTT

GlyGluAsnCysGlyTyrArgArgCysArgAlaSerGlyValLeuThrThrSerCysGly

6721 GGGGGGAGAACTGCGGCTATCGCAGGTGCGCGGAGCGGCTACTGACAACTAGCTGTG  
CCCCCTCTTGACGCGATAGCTCCAGGCGGCTCGCGCATGACTGTTGATCGACAC

AsnThrLeuThrCysTyrIleLysAlaArgAlaAlaCysArgAlaAlaGlyLeuGlnAsp

6781 GTAACACCTCACTTGTCTACATCAAGGCCCGGCGAGCTGTGAGCGCGAGGCTCCAGG  
CATTGTGGAGTGAACGATGTAGTTCCGGGCCGCTCGGACAGCTCGGCGTCCCGAGGTCC

CysThrMetLeuValCysGlyAspAspLeuValValIleCysGluSerAlaGlyValGln

6841 ACTGCACCATGCTCGTGTGTGGGACGACTAGTCTGTTATCTGTGAAGCGCGGGGGTCC  
TGACGTGGTACGAGCACACACGCTGCTGAATCAGCAATAGACACTTTCGCGCCCCAGG

FIG. 47-7

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ValGluIleArgGlnValGlyAspPheHisTyrValThrGlyMetThrThrAspAsnLeu  
 4921 ATGTGGAGATAAGGCAGGTGGGGGACTTCCACTACGTGACGGGTATGACTACTGACAATC  
 TACACCTCTATTCCGTCCACCCCTGAAGGTGATGCACTGCCCATACTGATGACTGTTAG  
  
 LysCysProCysGlnValProSerProGluPhePheThrGluLeuAspGlyValArgLeu  
 4981 TCAAATGCCCGTGCAGGTCCCATCGCCOGAATTTTTCACAGAATTGGACGGGGTGCGCC  
 AGTTTACGGGCAGGTCCAGGGTAGCGGGCTTAAAAAGTGTCTTAACCTGCCCCACGGGG  
  
 HisArgPheAlaProProCysLysProLeuLeuArgGluGluValSerPheArgValGly  
 5041 TACATAGGTTTGCGCCCTCTGCAAGCCCTTGTCTCGGGAGGAGGTATCATTACAGTAG  
 ATGTATCCAAACGCGGGGGACGTTCCGGGAACGACGCCCTCCTCCATAGTAAGTCTCATC  
  
 LeuHisGluTyrProValGlySerGlnLeuProCysGluProGluProAspValAlaVal  
 5101 GACTCCACGAATACCCGGTAGGGTGGCAATTACCTTGGAGCCGGAACCGGACGTGGCCG  
 CTGAGGTGCTTATGGGCCATCCAGCGTTAATGGAACGCTCGGGCTTGGCTGCACCGGC  
  
 LeuThrSerMetLeuThrAspProSerHisIleThrAlaGluAlaAlaGlyArgArgLeu  
 5161 TGTTGACGTCCATGCTCACTGATCCCTCCCATATAACAGCAGAGGCGCGCGGCGAAGGT  
 ACAACTGCAGGTACGAGTGACTAGGGAGGGTATATTGTGCTCTCGCGCGGCCGCTTCCA  
  
 AlaArgGlySerProProSerValAlaSerSerSerAlaSerGlnLeuSerAlaProSer  
 5221 TGGCGAGGGGATCACCCCTCTGTGGCCAGCTCCTCGGCTAGCCAGCTATCCGCTCCAT  
 ACCGCTCCCTAGTGGGGGAGACACCGGTCGAGGAGCCGATCGGTGATAGGCGAGGTA  
  
 LeuLysAlaThrCysThrAlaAsnHisAspSerProAspAlaGluLeuIleGluAlaAsn  
 5281 CTCTCAAGGCAACTTGCACCGCTAACCATGACTCCCTGTATGCTGAGCTCATAGAGGCCA  
 GAGAGTTCCGTTGAACGTGGCGATTGGTACTGAGGGGACTACGACTGAGTATCTCCGGT  
  
 LeuLeuTrpArgGlnGluMetGlyGlyAsnIleThrArgValGluSerGluAsnLysVal  
 5341 ACCTCCTATGGAGGCAGGAGATGGGCGCAACATCACCAGGGTTGAGTCAGAAAACAAAG  
 TGGAGGATACCTCCGCTCTTACCGCGCTTGTAGTGTCCCAACTCAGTCTTTTGTTC  
  
 ValIleLeuAspSerPheAspProLeuValAlaGluGluAspGluArgGluIleSerVal  
 5401 TGGTGAATCTGGACTCCTTCGATCCGCTTGTGGCGGAGGAGGACGAGCGGAGATCTCCG  
 ACCACTAAGACCTGAGGAAGCTAGGCGAACACCGCTCTCTCTGCTCGCCCTCTAGAGGC  
  
 ProAlaGluIleLeuArgLysSerArgArgPheAlaGlnAlaLeuProValTrpAlaArg  
 5461 TAACCGCAGAAATCCTGCGGAAGTCTCGGAGATTGCGCCAGGCCCTGCCGTTTGGGGCG  
 ATGGGCGTCTTTAGGACGCTTCAGAGCCTCTAAGCGGTTCCGGGACGGGCAACCCGCG  
  
 ProAspTyrAsnProProLeuValGluThrTrpLysLysProAspTyrGluProProVal  
 5521 GGCCGGACTATAACCCCGCTAGTGGAGACGTGGAAGGCGGACTACGAACCACTG  
 CCGCGTGATATGGGGGGGATCACCTCTGCACTTTTTCGGGCTGATGCTTGGTGGAC  
  
 ValHisGlyCysProLeuProProProLysSerProProValProProProArgLysLys  
 5581 TGGTCCATGGCTGTCCGCTTCCACCTCCAAAGTCCCTCCTGTGCTCGCCCTCGGAAGA  
 ACCAGGTACCGACAGGCGAAGGTGGAGTTTCAGGGGAGGACACGGAGGCGGAGCCTTCT  
  
 ArgThrValValLeuThrGluSerThrLeuSerThrAlaLeuAlaGluLeuAlaThrArg  
 5641 AGCGGACGGTGGTCTCACTGAATCAACCTATCTACTGCTTGGCCGAGCTCGCCACCA  
 TCGCCTGCCACAGGAGTGACTTAGTTGGGATAGATGACGGAACCGGCTCGAGCGGTGGT  
  
 SerPheGlySerSerSerThrSerGlyIleThrGlyAspAsnThrThrThrSerSerGlu  
 5701 GAAGCTTTGGCAGCTCTCAACTTCCGGCATTACGGGGACAAATACGACAACATCCTCTG  
 CTTTCGAAACCGTCGAGGAGTTGAAGGCCGTAATGCCCGCTGTTATGCTGTTGTAGGAGAC  
  
 ProAlaProSerGlyCysProProAspSerAspAlaGluSerTyrSerSerMetProPro  
 5761 AGCCCGCCCTTCTGGCTGCCCCCGACTCCGAOGCTGAGTCTATTCTCCATGCCCC  
 TCGGGCGGGGAAGACCGACGGGGGGCTGAGGCTGCGACTCAGGATAAGGAGGTACGGGG  
  
 LeuGluGlyGluProGlyAspProAspLeuSerAspGlySerTrpSerThrValSerSer  
 5821 CCTGGAGGGGAGCCTGGGGATCCGGATCTTAGCGACGGGTGATGGTCAAOGGTACAGTA  
 GGGACCTCCCCCTCGGACCCCTAGGCCAGTAATCGCTGCCAGTACCACTTGCCAGTCAAT  
  
 GluAlaAsnAlaGluAspValValCysCysSerMetSerTyrSerTrpThrGlyAlaLeu

FIG. 47-6

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GluAspAlaAlaSerLeuArgAlaPheThrGluAlaMetThrArgTyrSerAlaProPro  
 6901 AGGAGGACGCGCGAGCCTGAGAGCCTTCACGAGGCTATGACCAGGTACTCGCCCCCCC  
 TCCTCCTGCGCGCTCGGACTCTCGGAAGTGCTCGGATACTGGTCCATGAGGCGGGGG

GlyAspProProGlnProGluTyrAspLeuGluLeuIleThrSerCysSerSerAsnVal  
 6961 CTGGGGACCCCCACAACCAGAATACGACTTGGAGCTCATAACATCATGCTCCTCCAAAG  
 GACCCCTGGGGGGTGTGGTCTTATGCTGAACCTCGAGTATTGTAGTACGAGGAGGTGTC

SerValAlaHisAspGlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThrThr  
 7021 TGTGAGTCGCCCCACGACGGCGCTGGAAAGAGGGTCTACTACCTACCCGTGACCCACAA  
 ACAGTCAGCGGGTGCTGCGCGACCTTCTCCAGATGATGGAGTGGCACTGGGATGTT

ProLeuAlaArgAlaAlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeuGly  
 7081 CCCCCCTCGCGAGAGCTGCGTGGGAGACAGCAAGACCACTCCAGTCAATTCCTGGCTAG  
 GGGGGGAGCGCTCTCGACGCACCTCTGTCTCTGTGTGAGGTGAGTTAAGGACCGATC

AsnIleIleMetPheAlaProThrLeuTrpAlaArgMetIleLeuMetThrHisPhePhe  
 7141 GCAACATAATCATGTTTGCCCCACACTGTGGGCGAGGATGATACTGATGACCCATTCT  
 CGTTGTATTAGTACAAACGGGGGTGTACACCGCTCTACTATGACTACTGGGTAAAGA

SerValLeuIleAlaArgAspGlnLeuGluGlnAlaLeuAspCysGluIleTyrGlyAla  
 7201 TTAGCGTCCTTATAGCCAGGACCACTTGAACAGGCTCGATTGCGAGATCTACGGGG  
 AATCGCAGGAATATCGGTCCCTGGTGAACCTGTCCGGGAGCTAACGCTCTAGATGCC

CysTyrSerIleGluProLeuAspLeuProProIleIleGlnArgLeu  
 7261 CCTGCTACTCCATAGAACCACTTGATCTACCTCCAATCATTCAAAGACTC  
 GGACGATGAGGTATCTTGGTGAAC TAGATGGAGGTCTAAGTTTCTGAG

FIG. 47- 8